W1
AN452
CONTAINS INDEX
"CURR. LIST MED. LIT."

ANNALES MEDICINAE EXPERIMENTALIS ET BIOLOGIAE FENNIAE

NATIONAL MEDICINE
MAR 24 1959

REDACTORES:

E. MUSTAKALLIO (TURKU)
U. UOTILA (HELSINKI)
ARMAS VARTIAINEN (HELSINKI)
ALVAR WILSKA (HELSINKI)
A. I. VIRTANEN (HELSINKI)

EDITOR
K. O. RENKONEN

REDIGENDOS CURAVIT
ODD WAGER

VOL. 36 1958 FASC. 4

MERCATORIN KIRJAPAINO
HELSINKI, FINLAND

QLML
26 FEB 1959

Annales Medicinae Experimentalis et Biologiae Fenniae

is a direct continuation of the *Acta Societatis Medicorum Fennicae »Duodecim»*, 1919—1930 (Vols. I—XII) and the *Acta Societatis Medicorum Fennicae »Duodecim», Ser. A*, 1931—1946 (Vols. XIII—XXIV).

The journal is published by the Finnish Medical Society »Duodecim» with the object of providing an opportunity to publish articles on experimental medicine and on related biological subjects.

Each number of the journal contains 80—100 pages, four numbers forming one volume. Articles are accepted for publication with the understanding that they are original contributions never previously published. The manuscripts should be in English, French or German, and typewritten. They should not exceed a total length of 12 pages, and there should be a short summary at the end of the article.

The subscription price is Fmks 2000 per volume in Finland and $ 7 in foreign countries. More extensive works, published as supplements, the subscribers receive free of charge.

Address for subscription, exchange of reviews, and all communications:

Annales Medicinae Experimentalis et Biologiae Fenniae

Kalevankatu 11, Helsinki, Finland.

Address for exchange of publications: Snellmaninkatu 9—11, Helsinki, Finland.

Annales Medicinae Experimentalis et Biologiae Fenniae

est une suite directe des revues *Acta Societatis Medicorum Fennicae »Duodecim»*, 1919—1930 (V. I—XII) et *Acta Societatis Medicorum Fennicae »Duodecim»*, Ser. A, 1931—1946 (V. XIII—XXIV).

La revue est éditée par la Société de médecins finnois »Duodecim», et a pour but d'offrir l'occasion de publier des recherches scientifiques appartenant à la médicine expérimentale et à la biologie étroitement liée avec la médicine.

La revue paraît en cahiers comprenant à peu près 80—100 pages. Quatre cahiers forment un volume. Les articles destinés à la revue ne doivent pas être publiés ailleurs. Les manuscrits dactylographiés doivent être rédigés en français, allemand ou anglais et leur longeur totale ne doit pas en général dépasser 12 pages. Un court résumé doit se trouver à la fin.

Le prix de l'abonnement pour la Finlande est marcs 2000, pour l'étranger $ 7 par volume. Les travaux plus étendus, qui seront éventuellement publiés en suppléments, seront distribués gratuitement aux abonnés.

Adresse pour abonnement, échange de journaux et toutes autres communications:

Annales Medicinae Experimentalis et Biologiae Fenniae

Kalevankatu 11, Helsinki, Finlande.

Address pour échange de journaux: Snellmaninkatu 9—11, Helsinki, Finlande.

Annales Medicinae Experimentalis et Biologiae Fenniae

sind eine direkte Fortsetzung der *Acta Societatis Medicorum Fennicae »Duodecim»*, 1919—1930 (Vol. I—XII) und der *Acta Societatis Medicorum Fennicae »Duodecim», Ser. A*, 1931—1946 (Vol. XIII—XXIV).

Die Zeitschrift wird vom *Finnischem Ärzteverein »Duodecim»* herausgegeben und hat zur Aufgabe wissenschaftliche Untersuchungen aus dem Gebiete der experimentellen Medizin und sich daran schliessenden biologischen Forschungsgebieten aufzunehmen.

Jede Nummer der Zeitschrift erscheint in einem Umfange von 80—100 Druckseiten. Vier Nummern bilden ein Volumen. In die Zeitschrift werden nur Originalarbeiten aufgenommen, die nicht früher veröffentlicht worden sind. Die mit Schreibmaschiene geschriebenen Manuskripte sind in deutscher, englischer oder französischer Sprache einzusenden und sollen im allgemeinen nicht mehr als 12 Druckseiten betragen. Jede Untersuchung ist durch eine kurze Zusammenfassung abzuschliessen.

Der Bezugspreis beträgt für das Innland 2000 Fmk und fürs Ausland 7 $. Umfangreichere Untersuchungen, die als Supplemente herausgegeben werden können, erhalten die Abonnenten abgabefrei.

Bezugsadresse sowie Anschrift für Austausch von Zeitschriften und alle anderen Mitteilungen:

Annales Medicinae Experimentalis et Biologiae Fenniae

Kalevankatu 11, Helsinki, Finnland.

Anschrift für Austausch von Zeitschriften: Snellmaninkatu 9—11, Helsinki, Finnland.

autonomisen hermeston

MILTERGAL

Tabl. 20, 50, 100

autonomisen hermoston dysfunktioon

Oy Medica Ab

Uusi vegetatiivista hermostoa rauhoittava valmiste

MILTERGAL tabl.

Meprobamat.	0,2
Dihydroergocristin. isethion.	0,0002
Butylscopol. bromid.	0,005
Sacch. obd.	

INDIKAATIOT: Vegetatiivisen dystonian ja neuroosin eri muodot.

ANNOSTUS: 1—2 tabl. 2—3 kertaa päivässä.

Vuodesta 1948 alkaen Medica on valmistanut vegetatiivisen hermoston rauhoittamiseen tarkoitettuja Ergatral tabletteja. Sama periaate, yhdistää samaan valmisteeseen sympaattisen hermoston toimintoja rauhoittava sekalealkaloidi, parasympaattisen hermoston toimintoja rauhoittava atropiiniryhmän alkaloidi sekä keskushermoston toimintoja ja vegetatiivisia keskuksia rauhoittava lääkeaine, on edelleenkin yleisesti hyväksytty. Lääkeaineiden alalla viime vuosina tapahtunut voimakas kehitys on tuonut aikaisemmin käytännössä olleiden aineiden tilalle monessa suhteessa sopivampia lääkeaineita. Niinpä **dihydroergokristiinin** sympatikolyyttinen vaikutus on useita kertoja voimakkaampi, mutta sen myrkyllisyys huomattavasti pienempi kuin ergotamiinin ja se ei ergotamiinin tavoin supista kohdun eikä verisuonien sileätä lihaksistoa.

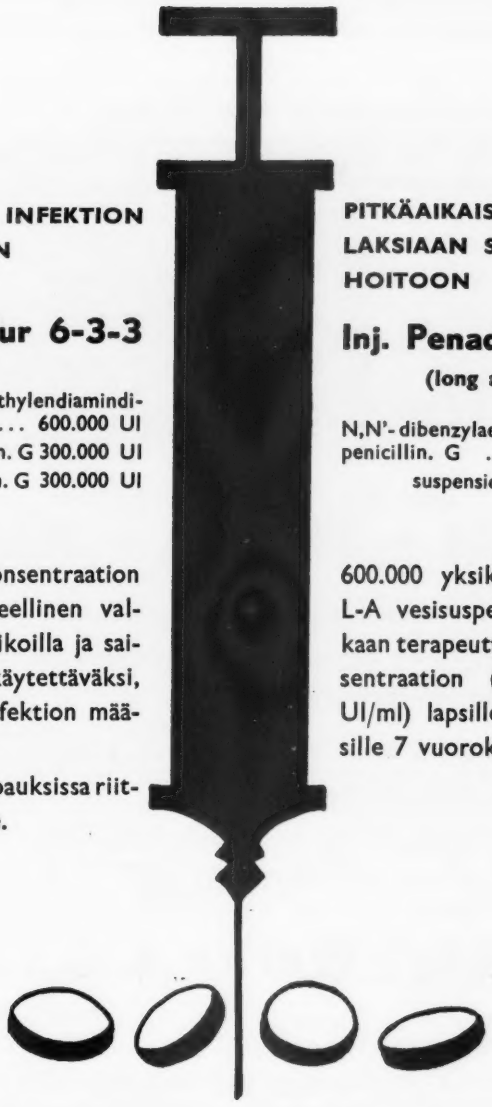
Dihydroergokristiinilta, joka päinvastoin laajentaa jossain määrin perifeeristä verisuonistoa, puuttuvat siis ne vaikutukset, jotka ergotamiinia käytettäessä voivat aiheuttaa komplikaatioita.

Butylskopolamiinilla on atropiininkaltaiset vaikutukset parasympaattiseen hermostoon. Näitä tehostavat sen autonomisia ganglioita salpaavat vaikutukset. Sen etuna atropiiniin verrattuna on, että sen vaikutukset syljenerityksen ja sydämen lyöntitiheyteen ovat huomattavasti heikommat. Butylskopolamiinilla ei ole sanottavia vaikutuksia keskushermostoon ja sen myrkyllisyys on atropiiniin ja skopolamiiniin verrattuna pieni.

Meprobamaatti laukaisee psyykkisiä ja fyysisiä jännitystiloja ja vaikuttaa rauhoittavasti silti aiheuttamatta väsymystä, minkä vuoksi sitä on suuressa mitassa ruvettu käyttämään sedatiivina (ataraktina) varsinkin barbituraattien asemasta. Sen toksisuus on n. 5 kertaa pienempi kuin fenemaalin. Eräissä tapauksissa sen on todettu aiheuttavan allergisia ihoreaktioita.

Miltergal on kokoomuksensa perusteella erittäin sopiva lääke hoidettaessa ambulatorisesti sellaisia sairauksia, joiden syynä on tai joihin liittyy autonomisen hermoston dysfunktio.

Medica



ENSIAVUKSI INFEKTION TORJUNTAAN

Inj. Penadur 6-3-3

N,N'-dibenzylaethylendiamindi-penicillin. G ..... 600.000 UI
Procain. penicillin. G 300.000 UI
Kalium penicillin. G 300.000 UI

Suuren alkukonsentraation ansiosta ihanteellinen valmiste poliklinikoilla ja sairaskäynneillä käytettäväksi, kun tarkka infektion määritys puuttuu.

Useimmissa tapauksissa riittää yksi ruiske.

PITKÄAIKAISEEN PROFYLAKSIAAN SEKÄ JATKOHOITOON

Inj. Penadur L-A

(long acting)

N,N'-dibenzylaethylendiamindi-penicillin. G .... 300.000 UI
suspensio aquosa

600.000 yksikköä Penadur L-A vesisuspensiota saa aikaan terapeuttisen verikonsentraation (vähint. 0,05 UI/ml) lapsille 14 ja aikuisille 7 vuorokaudeksi.

NOPEASTI KORKEA ALKUKONSENTRAATIO MYÖS PERORAALISESTI

V-Penicillin "500" tabl. —

Phenoxymethylpenicillin. kal. 500.000 UI

Liukenee vasta suolistossa. Nopean imeytymisen ansiosta korkea alkukonsentraatio.

Wyeth

Leiras

ATLAS — UNIVERSAL — OKSIMETRI

soveltuu korvakappaleen avulla arteriaalisen veren happikyllästysasteen tarkkailuun sekä kyvettien avulla verinäytteen hemoglobiinin määräämiseen.

Käytössä oksimetri on osoittautunut käyttövarmaksi sekä tarkat mittaustulokset antavaksi.

Valmistaja:

Atlas—Werke A. G., Bremen

Pääedustaja Suomessa:

LÄÄKINTÄSÄHKÖ Oy

MIKONKATU 13 A, HELSINKI

PUH. 11 401

FROM THE ZOOLOGICAL MUSEUM, UNIVERSITY OF HELSINKI, FINLAND

SOME PECULIARITIES OF THE SEASONAL OCCURRENCE OF POLIOMYELITIS IN FINLAND

by

PEKKA NUORTEVA

(Received for publication April 11, 1958)

In the temperate climates poliomyelitis is prevalent in late summer and spring. This epidemiological behaviour is so far poorly understood as the wintertime freezing does not inactivate the poliomyelitis virus but the desiccation at the elevated summer temperatures would do it. The assumption, that flies or other arthropods would be transmitters of poliomyelitis during the epidemics in summer could offer an explanation for the epidemiological behaviour of this disease. In fact, viruses of poliomyelitis have also in many cases been extracted from house flies and from several species of blowflies collected during or before epidemics (1, 2, 4, 5, 7, 8, 9, 11, 12). Poliomyelitis viruses may survive at least three weeks in the blowfly *Phormia regina* Meig. (3). The role of insects is however not generally regarded as an dominant element in the spread of this disease.

In the present article, the attention is drawn to some peculiar features in the seasonal occurrence of poliomyelitis in Finland and an attempt is made to elucidate these peculiarities from an entomologists point of view.

MATERIAL

The evidence for the seasonal occurrence of poliomyelitis in Finland is based on cases which have been reported to the State Medical Board during the years 1938—1957. Before the year 1953

no distinction was made between the paralytic and nonparalytic cases. In this investigation only paralytic cases are considered for the period 1953—1957. For the period 1938—1952 all reported cases are considered but it is likely that only few cases reported during these years may have been nonparalytic ones.

Notes on the flying times and geographic distribution of some flies are based on the notes of the trapping dates and of the localities of these insects in the Entomological Museum of the University of Helsinki. The series examined for this paper consisted of more than 1000 specimens of the genus *Lucilia* collected by different persons in 95 parishes situated in various parts of Finland. A more detailed report on this series will be published in Annales Entomologici Fennici.

RESULTS AND DISCUSSION

The monthly occurrence of paralytic poliomyelitis cases in the different provinces of Finland during the years 1938—1957 is given in Table 1 and in Figures 1 and 2. In the latter figure is given the geographic site of the different provinces and the day for the mean occurrence of poliomyelitis in the different provinces.

From these data it is to observe, that the morbidity in paralytic poliomyelitis shows its minimum in the month february, increases then slightly until june, shows a more rapid increase until september and october after which a gradual decrease follows. The course of seasonal morbidity is not strictly similar in all provinces.

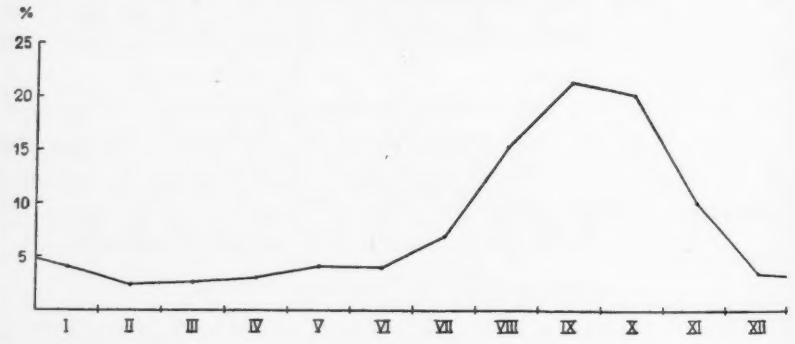


Fig. 1. — The seasonal occurrence of paralytic poliomyelitis in Finland during the years 1938—57.

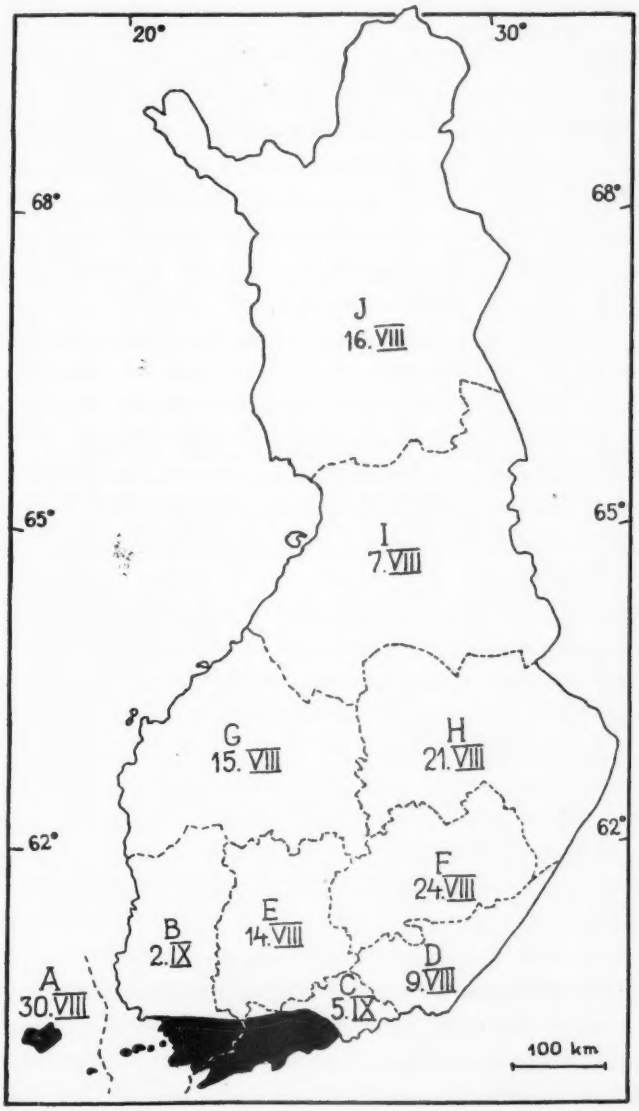


Fig. 2. — The geographical site of the different provinces in Finland (the letter symbols for the different provinces are given in the table 1) and the days for the mean occurrence of poliomyelitis in the different provinces. The black area indicates the area in which the blowflie *Lucilia sericata* does occur. — Observe the late days for the mean occurrence of poliomyelitis in this area.

TABLE 1

SEASONAL OCCURRENCE OF PARALYTIC POLIOMYELITIS IN THE DIFFERENT PROVINCES IN FINLAND DURING THE YEARS 1938—1957

Province	Letter Symbol	Reported Cases in Numbers per Month												Σ
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Ahvenanmaa	A	1	—	2	1	—	1	—	2	14	4	4	1	30
Turku & Pori	B	28	15	12	19	21	27	40	127	217	209	97	38	850
Uusimaa	C	33	18	23	21	20	38	63	167	271	275	147	71	1147
Kymi.....	D	20	12	19	16	28	22	22	68	68	72	54	20	421
Häme	E	58	21	22	37	42	38	70	131	233	179	81	58	970
Mikkeli	F	12	13	8	13	12	13	27	77	81	68	68	12	404
Vaasa	G	38	30	25	20	44	25	89	106	106	149	121	40	793
Kuopio	H	20	14	25	24	36	37	59	141	156	135	58	32	737
Oulu	I	24	18	19	19	25	23	30	66	96	84	37	26	467
Lappi	J	9	2	5	9	8	16	16	40	38	41	14	13	211
Whole country		243	143	160	179	236	240	416	925	1280	1216	681	311	6030
		Reported Cases in Percentages per Month												
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Ahvenanmaa	A	3.3	—	6.7	3.3	—	3.3	—	6.7	46.7	13.3	13.3	3.3	
Turku & Pori	B	3.3	1.8	1.4	2.2	2.5	3.2	4.7	14.9	25.5	24.6	11.4	4.5	
Uusimaa	C	2.9	1.6	2.0	1.8	1.7	3.3	5.5	14.6	23.6	24.0	12.8	6.2	
Kymi.....	D	4.8	2.9	4.5	3.8	6.7	5.2	5.2	16.2	16.2	17.1	12.8	4.8	
Häme	E	6.0	2.2	2.3	3.8	4.3	3.9	7.2	13.5	24.0	18.5	8.4	6.0	
Mikkeli	F	3.0	3.2	2.0	3.2	3.0	3.2	6.7	19.1	20.1	16.8	16.8	3.0	
Vaasa	G	4.8	3.8	3.2	2.5	5.6	3.2	11.2	13.4	13.4	18.8	15.3	5.0	
Kuopio	H	2.7	1.9	3.4	3.3	4.9	5.0	8.0	19.1	21.2	18.3	7.9	4.3	
Oulu	I	5.1	3.9	4.1	4.1	5.4	5.0	6.4	14.1	20.6	18.0	7.6	5.6	
Lappi	J	4.3	1.0	2.4	4.3	3.8	7.6	7.6	19.0	18.1	19.4	6.9	6.2	
Whole country		4.0	2.4	2.7	3.0	3.9	4.0	6.9	15.3	21.2	20.2	11.3	5.2	

It is to note, that this disease occurs significantly later in the provinces of Ahvenanmaa, Turku & Pori and Uusimaa at the southern coastal area of Finland than it occurs in the other parts of this country. The day for mean occurrence of paralytic poliomyelitis in the southern coastal area is 4. IX. Outside this area the respective day is 15. VIII. Partly the differences in the days for mean occurence derive from the fact, that poliomyelitis is more common during winter in the northern parts than it is in the south of Finland. A difference exist also in the seasonal occurrence during the epidemic time (see Fig. 3). In Helsinki the peak of the epidemy occurs one

month later than in the other parts of Finland (Fig. 3). This relationship is peculiar since the areal in which poliomyelitis occurs latest in the season is the southernmost and warmest part of Finland and is also an area, where the human population is in general densiest. A early seasonal occurrence and rapid spreading of poliomyelitis would thus be expected for this area if it is assumed that poliomyelitis spreads by contact between individuals. If flies would be the dominant element in the spread of poliomyelitis it would be possible to explain this peculiar epidemiological feature. The coastal area, in which poliomyelitis occurs exceptionally late in the season, is namely the only area in Finland where the blowflie *Lucilia* (*Phænicia*) *sericata* Meig. does occur (Fig. 2). The paramount greatest abundance this flie shows in the city Helsinki. *Lucilia sericata* is a flie, from which the american investigators have most often obtained poliomyelitis viruses and from which viruses have been obtained also before the outbreak of an epidemic of this disease (1, 2, 10). The flying time of this species in Finland is given in Fig. 4. It is to observe that the seasonal occurrence of this flie during the warm season of the year is very similar with that of poliomyelitis. This flie would thus have the possibility to function as a carrier of poliomyelitis in the southern coastal area of Finland. That would not indeed exclude even other flies to act as carriers in the south. Other flies with similar habits must also be assumed to act as carriers in the northern parts of Finland where *Lucilia sericata* does not live. No other flie, which comes in question as a carrier of poliomyelitis viruses has the maximum of seasonal abundance as late as it has *Lucilia sericata*. If flies would act as an essential element in the spread of poliomyelitis the late occurrence of poliomyelitis in the south would be understable to be influenced by the late flying of *Lucilia sericata*.

The fact, that poliomyelitis occurs more commonly during winter in the northern provinces than in the south is also an interesting and significant feature in the epidemiology of paralytic poliomyelitis in Finland (Table 1 and Fig. 3). Indeed it could be assumed that the couldiness of the climate would in some way increase the intime contact between the individuals. In general the physicians dont believe, that arthropods would transmit poliomyelitis during winter. This comprehension has been one of the heaviest arguments against the hypothesis of arthropod-transmission of

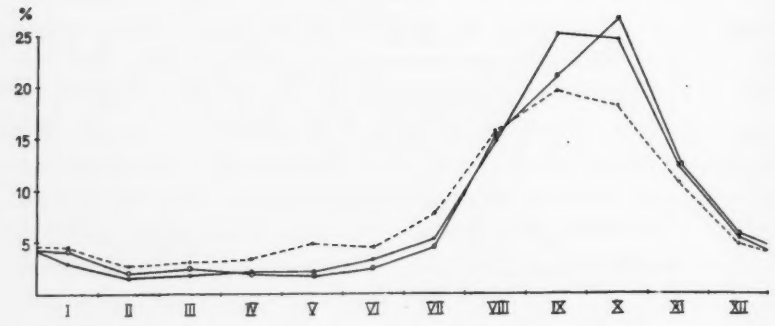


Fig. 3. — The seasonal occurrence of paralytic poliomyelitis in the city Helsinki (—o—o—), in the provinces Ahvenanmaa, Turku & Pori and Uusimaa at the south coast (—·—·—) and in the other provinces in Finland (· – – · – – ·).

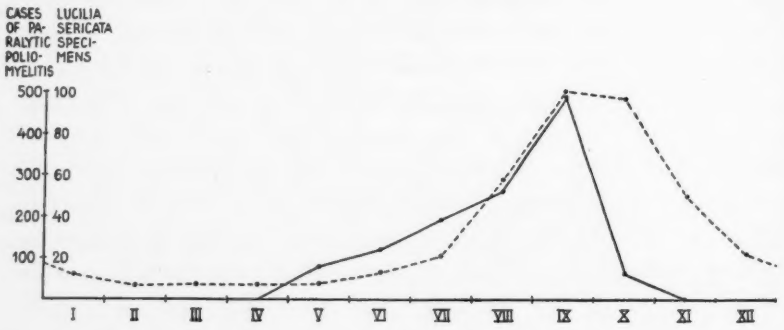


Fig. 4. — The seasonal abundance of the blowflie *Lucilia sericata* in Finland (———) and the seasonal occurrence of paralytic poliomyelitis cases (– – – – –) in the provinces inhabited by this species.

poliomyelitis. From an entomologists point of view a transmission by arthropods seems not to be quite impossible. There are many species of flies, which overwinter in houses and are sometimes active indoors also during winter. Melnick (1) has shown, that the flie *Cynomyopsis cadaverina* R. D. harbors viruses also very late in autumn and remarks, that the virus could possibly overwinter in this flie. Flies will survive the winter better in houses without central heating as this mode of heating decreases the relative humidity of air in such degree that the flies most often die during 2—3 months. The percentage of centralheated houses is significantly lesser in the northern provinces of Finland than in the southern ones.

If the overwintering flies would play some role in the epidemiology of poliomyelitis, a more common occurrence of this disease would thus exist in the northern provinces of Finland — which as already mentioned, holds true.

It has recently been shown in many instances (6), that cockroaches harbor the poliomyelitis virus in their alimentary tracts. These inquilines occur more commonly in the northern provinces of Finland than in the southern ones. Thus the more common occurrence of poliomyelitis during winter in the northern parts of this country could possibly be explained by means of these insects as well as by means of overwintering flies. — Until yet it exist, however, no evidence on the significance of cockroaches or overwintering flies in the epidemiology of poliomyelitis, but they are without doubt worth of particular attention in this respect.

SUMMARY

In the present article, data are given on the seasonal occurrence of paralytic poliomyelitis in the different provinces of Finland. In three provinces at the southern coastal area poliomyelitis occurs somewhat later than in the other provinces. This fact can be correlated to the occurrence of the virus-spreading blowflie *Lucilia sericata* Meig., which occurs only in the southern coastal area and which shows a seasonal maximum of abundance later in the season than other flies. The more common occurrence of poliomyelitis in winter in the northern provinces than in the south is discussed in connection of a hypothetical transmission of the disease by overwintering flies and cockroaches.

REFERENCES

1. Melnick, J. L.: Amer. Journ. Hyg. 1949:*49:*8.
2. Melnick, J. L., and Dow, R. P.: Amer. Journ. Hyg. 1953:*58:*288.
3. Melnick, J. L., and Penner, L. R.: Proc. Soc. Exper. Biol. Med. 1947:*65:*342.
4. Melnick, J. L., and Ward, R.: Journ. Inf. Diseases 1945:*77:*249.
5. Paul, J. R., Trask, J. D., Bishop, M. B., Melnick, J. L., and Casey, A. E.: Science 1941:*94:*395.
6. Roth, L. M., and Willis, E. R.: Smithsonian Miscell. Collections 1957:*134:*1.

7. Sabin, A. B., and Ward, R.: Science 1941:*94:*590.
8. Sabin, A. B., and Ward, R.: Science 1942:*95:*300.
9. Toomey, J. A., Takacs, W. S., and Tischer, L. A.: Proc. Soc. Exper. Biol. Med. 1941:*48:*637.
10. Trask, J. D., Paul, J. R., and Melnick, J. L.: Journ. Exper. Med. 1943:*77:*531.
11. Trask, J. D., and Paul, J. R.: Journ. Exper. Med. 1943:*77:*545.
12. Ward, R., Melnick, J. L., and Horstman, D. M.: Science 1945:*101:* 491.

FROM THE DEPARTMENTS OF PHARMACOLOGY, UNIVERSITIES OF TURKU AND HELSINKI

THE EFFECT OF RESERPINE ON VASCULAR CHANGES PRODUCED BY TOBACCO SMOKING[1]

by

ESKO HISALO and N. T. KÄRKI

(Received for publication April 21, 1958)

Nicotine is regarded as the reason for the vascular changes produced by smoking (15). These reactions, *i.e.*, the constriction of skin vessels and the increase of heart rate and blood pressure, can be considered to be the result of the ability of nicotine to stimulate the adrenergic system as, for instance, the sympathetic ganglia and the adrenal medulla. Reserpine and some other Rauwolfia alkaloids are able to cause the release of noradrenaline from tissues (2, 8). Reserpine-treated animals do not react with the usual blood pressure increase to sympathetic stimulation (5). Nicotine fails to cause vasoconstriction in the perfused rabbit ear if the animal is previously treated with reserpine (14).

In view of the ability of reserpine to deplete noradrenaline from the tissues, we have studied whether reserpine treatment in human beings can alter the circulatory changes caused by tobacco smoking.

TEST ARRANGEMENTS

The test subjects were 18 healthy medical students and physicians aged from 22 to 36 years. Both sexes were represented, there being 14 males and 4 females. Ten of the test persons were habitual smokers but they had not smoked for 12—18 hours before the test. Measurements of the heart rate, blood pressure and cutaneous temperature were made in the forenoon before lunch.

[1] This work was supported by a grant from the Sigrid Jusélius Foundation.

To keep the conditions constant, the tests were conducted in a thermoregulated room, in the temperature of 23 ± 0.5°C. Relative humidity was between 50 and 55 per cent. During the experiment the subjects wore their usual indoor clothing, but both feet were exposed. They were in a half-recumbent, comfortable position and were allowed to read during the test. The thermometer (Thermorapid) electrode was connected to the tip of one great toe. Toe temperature, heart rate and blood pressure were registered at 5-minute intervals. For blood pressure measurement the ordinary Riva-Rocci sphygmomanometer was used.

When the subjects had been under observation for at least 30 minutes, during which the blood pressure and heart rate became stable, they were asked to smoke one standard non-filter cigarette in their own customary manner. Non-smokers were instructed to inhale. The smoking was finished in 6 to 8 minutes. Observations were continued until 60 minutes from the beginning of smoking.

The same measurements were repeated under reserpine treatment. The daily amount of reserpine was 1.0—1.5 mg divided into three or four doses and the treatment was continued for 3 or 4 days. As a rule the last dose of reserpine [1] was taken in the morning before the test.

RESULTS

Control Tests. — When evaluating the changes produced by smoking the mean of the last three readings before smoking was taken as the standard value. With these values were compared the highest heart rate and blood pressure readings and the lowest skin temperature observed after smoking.

Smoking caused in all the persons a decrease in the temperature of the toe, a faster heart rate and higher blood pressure. A typical response to smoking is shown in Fig. 1. In most cases the heart rate and the blood pressure increased almost immediately after smoking was begun and reverted to the pre-smoking level in 15 to 20 minutes. The skin temperature decrease was also observed already at the first measurement and it continued to fall during the first 15 to 20 minutes. The lowest temperature was reached in about 50 minutes after the commencement of smoking.

In Fig. 2 are shown the reactions of all the test subjects. The fall in the cutaneous temperature in some cases was considerable, being up to 9°C. The average toe temperature, which before smoking was 28.7, declined to 24.1°C.

1 We are indebted to Lääke Oy., Manufacturing Chemists, Turku, for reserpine tablets.

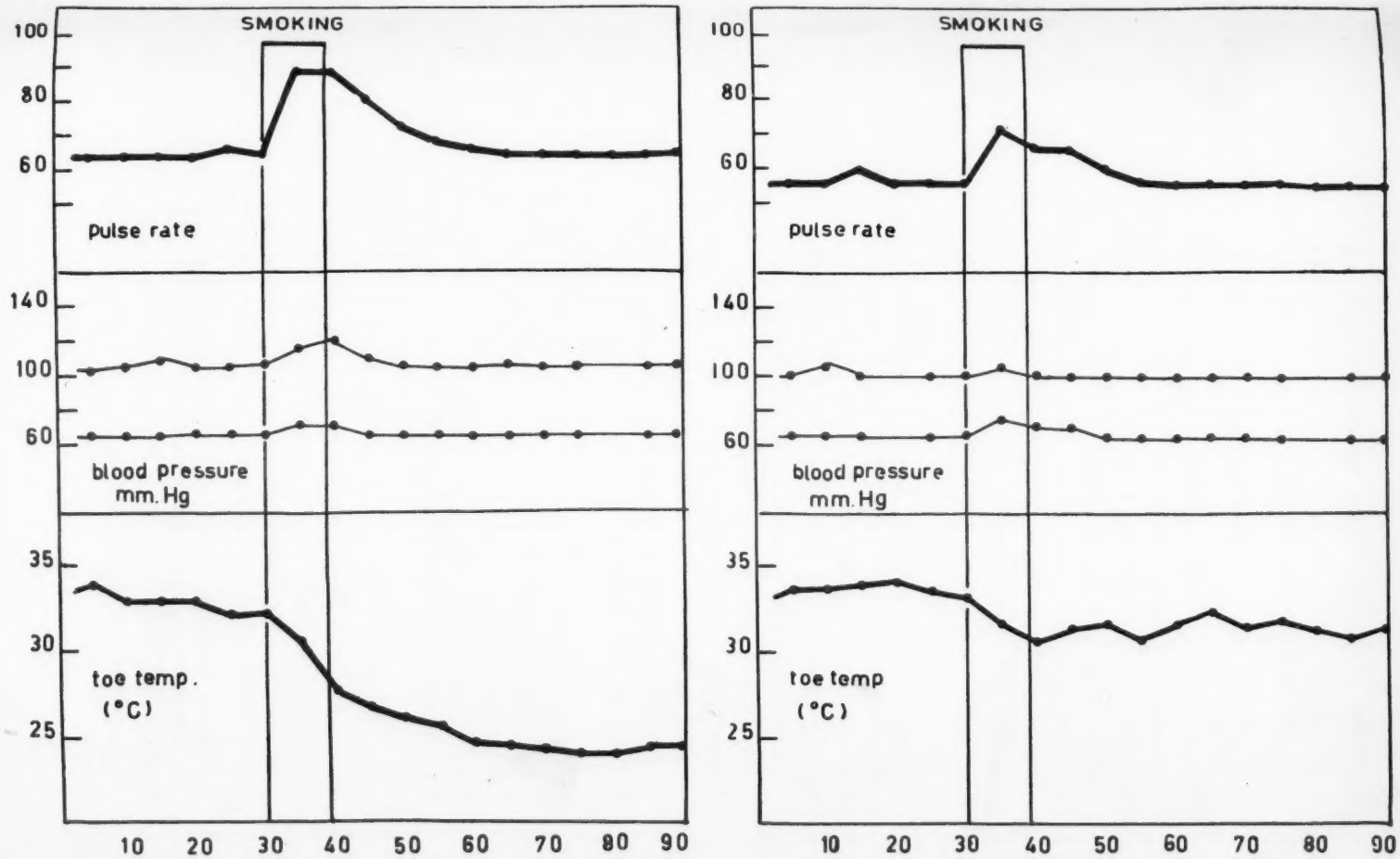


Fig. 1. — 22-year-old female test subject. Smoking induced changes in heart rate, blood pressure and cutaneous temperature before and during reserpine treatment. Abscissa: time in minutes.

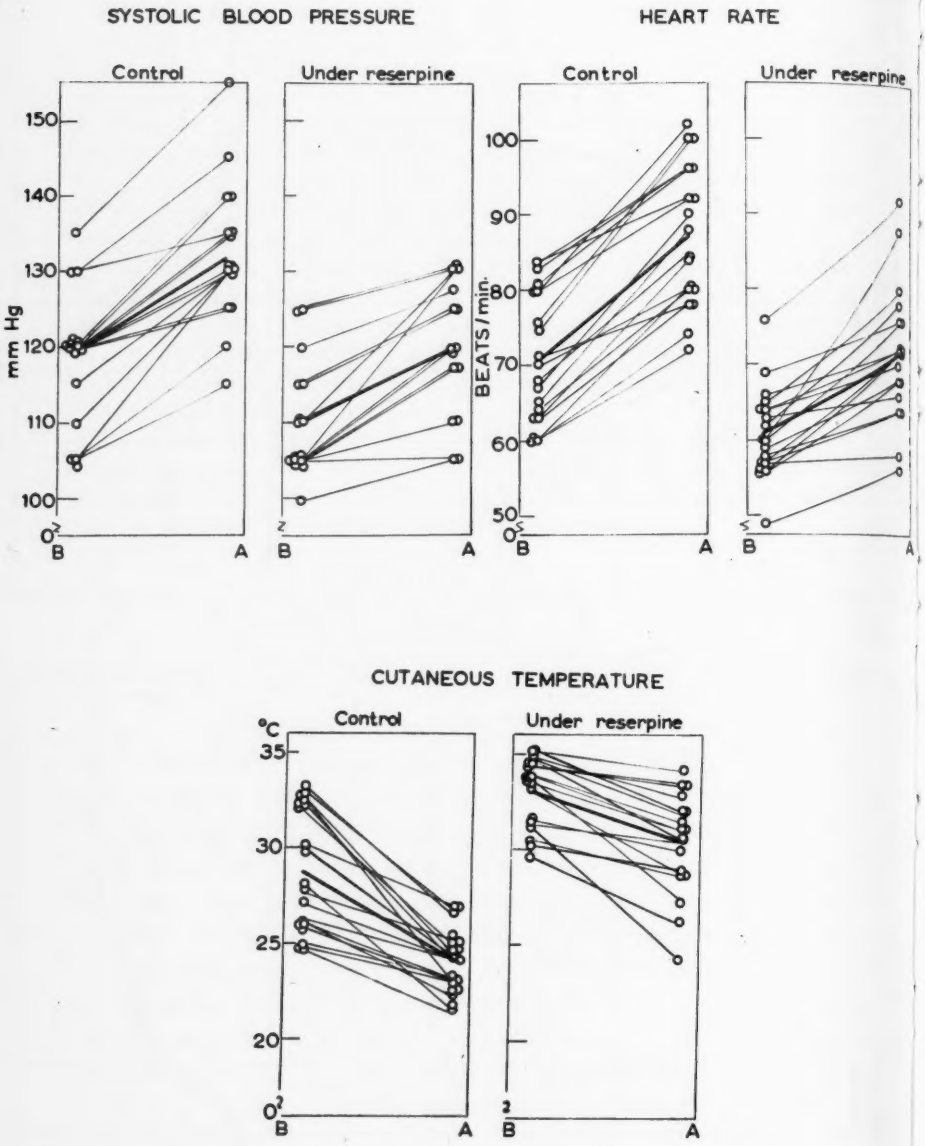


Fig. 2. — Changes in heart rate, blood pressure and cutaneous temperature in control experiments and under the influence of reserpine. Values of each test person before (B) and after smoking (A) are joined. Mean values are marked by heavy lines.

Smoking increased the mean heart rate from 70.6 to 87 beats/min. and the mean systolic blood pressure from 118 to 132 mm Hg.

Effects of Reserpine on Test Subjects. — It was found in preliminary experiments that the pharmacological effects characteristic to reserpine became manifest three to four days after commencement of the treatment. The majority of test subjects had subjective symptoms, such as lassitude and fatigue, nasal congestion, gastrointestinal disturbances, etc.

The heart rate averaged 61 beats/min., which was 9 beats/min. lower than value registered before smoking in the control experiments. The average systolic blood pressure also declined from 118 to 110 mm Hg.

The effect of reserpine was especially clear in the skin temperature. With the exception of one case the temperature had increased. The maximum increase was 9°C and the average 5.7°C. It was also found that when the feet were exposed before smoking the skin temperature had no tendency whatsoever to decrease.

Smoking under the Influence of Reserpine. — Fig. 1 shows one typical reaction to smoking after reserpine treatment. The decrease of tobacco-induced responses as compared with the control values is clearly seen. The individual smoking reactions in the heart rate, blood pressure and skin temperature are shown in Fig. 2, which also gives the mean values.

The mean increase in the heart rate was 11 beats/min., whereas in control tests with the same persons the increase was 16 beats/min. 13/18 persons showed smaller responses after reserpine and in three experiments there were no changes. When the results were examined by the so-called sign test the decrease in the heart rate was found to be statistically significant ($P<0.01$).

Without reserpine the mean increase of blood pressure was 14 mm Hg and after reserpine 9 mm Hg ($P>0.05$).

As mentioned above the skin temperature before smoking was 5.7°C higher after reserpine than in the control tests. This could induce increased heat dissipation. It was nevertheless found that in most cases (13/18) the temperature decreases caused by smoking were smaller than in control tests. On the average, decrease in skin temperature after smoking was 4.6°C in control experiments and 2.6°C during reserpine treatment. The result was statistically

significant (P~0.05). In control tests the decreased temperature lasted for an average of 50 minutes from the beginning of smoking, whereas after reserpine the lowest temperature was registered in about 15 minutes.

One of the test subjects had nausea from smoking. The same symptom appeared in her also during smoking after reserpine.

DISCUSSION

When relatively small amounts of nicotine are used, its vascular effects are ascribed primarily to stimulation of the vasomotor centre (7), chemoreceptors (17), the adrenal medulla (16) and the sympathetic ganglia (10, 11, 13) Reserpine produces adrenergic depression both centrally and peripherally, which is assumed to be due to the lack of adrenergic transmitter substances (5). Therefore it can be understood that the ability of nicotine to stimulate the sympathetic system is decreased under reserpine. Reserpine has also been shown to possess peripheral sympathetic inhibiting properties (1). It is not known whether this may contribute to the diminished smoking reaction. On the other hand, it has been shown that reserpine-treated animals are sensitised to noradrenaline (5).

It has been proved by Krayer and Paasonen (9, 12) and Carlsson *et al.* (5) that even rather small doses of reserpine cause a decreased noradrenaline content in the tissues. Using peroral administration Burger (3) showed a definite drop of noradrenaline in human blood after a three days' reserpine treatment. These observations support the assumption that our dosage (1.0—1.5 mg/day) of reserpine given during three to four days was sufficient to decrease the amount of adrenergic agents in the organism.

In the present experiments the temperature decrease caused by smoking lasted for a considerable time in the control experiments but not under the influence of reserpine. This prolonged decrease was apparently caused in part by the rather low environmental temperature. The nicotine-induced vasoconstriction was further maintained in a reflex manner by cooling of the skin (6).

It is well to remember in this connection the findings, that smoking causes secretion of the posterior pituitary antidiuretic hormone (4) and this may affect the cutaneous vessels.

It is known that the habitual use of tobacco is sometimes followed by vascular disorders and that the sympathectomy at

least partly improves this state. In view of the present findings, the use of reserpine in these disturbances as well as in conditions similar to Raynaud's disease may be justified.

SUMMARY

It was confirmed in experiments with 18 healthy subjects that the smoking of one cigarette increased the heart rate and the systolic blood pressure and decreased the cutaneous temperature of the foot.

The oral administration of 1.0—1.5 mg/day of reserpine for 3 to 4 days was followed, in addition of the heart rate and blood pressure decrease, by a significant rise in the skin temperature.

Smoking increased the heart rate and lowered the cutaneous temperature significantly less under reserpine treatment than in the control experiments without reserpine.

REFERENCES

1. Åblad, B.: Acta Pharm. Toxicol. 1957:*13:*213.
2. Bertler, Å., Carlsson, A., and Rosengren, E.: Naturwissenschaften 1956:*22:*521.
3. Burger, M.: Helv. Physiol. Acta 1956:*14:*C 13.
4. Burn, J. H.: Brit. Med. J. 1951:*II:*199.
5. Carlsson, A., Rosengren, E., Bertler, Å., and Nilsson, J.: Psychotropic Drugs, ed. Garattini, S., and Ghetti, V., Elsevier, Amsterdam 1957: p. 363.
6. Eckstein, J. W., Wood, J. E., and Wilkins, R. W.: Am. Heart J. 1957:*53:*455.
7. Hoskins, R. G., and Ranson, S. W.: J. Pharmacol. & Exper. Therap. 1915:*7:*375.
8. Holzbauer, M., and Vogt, M.: J. Neurochem. 1956:*1:*8.
9. Krayer, O., and Paasonen, M. K.: Acta Physiol. Scand. 1957:*42* suppl. *145:*88.
10. Langley, J. N., and Dickinson, W. L.: J. Physiol. 1890:*11:*297.
11. Langley, J. N., and Dickinson, W. L.: Proc. Roy. Soc. London 1889:*46:*423.
12. Paasonen, M. K., and Krayer, O.: Fed. Proc. 1957:*16:*1281.
13. Paton, W. D. M., and Perry, W. l. M.: J. Physiol. 1953:*119:*43.
14. Rand, M. J., and Burn, J. H.: J. Physiol. 1958:*140:*P 7.
15. Roth, G. M., McDonald, J. B., and Sheard, C.: J. A. M. A. Therap. 1944:*125:*761.
16. van Slyke, G. B., and Larsson, P. S.: J. Pharmacol. & Exper. Therap. 1950:*98:*400.
17. Wright, S.: J. Pharmacol. & Exper. Therap. 1935:*54:*1.

FROM THE DEPARTMENT OF ANATOMY, UNIVERSITY OF TURKU

EFFECT OF DEHYDRATION AND REHYDRATION ON THE ACID PHOSPHATASE ACTIVITY OF THE HYPOTHALAMIC MAGNOCELLULAR NUCLEI

by

U. K. RINNE and E. KIVALO

(Received for publication April 22, 1958)

According to the most recent investigations the magnocellular nuclei of the diencephalon are the place of origin of the hormones of the hypophysial posterior lobe. Histologically, this could be established by demonstrating that so-called neurosecretory activity occurs in the said nuclei (12).

The neurosecretory system has lately been investigated, for instance, by means of various dehydration and rehydration tests, the objective being that of throwing further light on the activity of the cells in the supraoptic nucleus and the paraventricular nucleus of the hypothalamus (e.g. 10, 5, 1, 2). Numerous investigations have shown that intensive metabolic processes take place in the cells of the above-mentioned nuclei (13). Eränkö, in 1951 in his investigation on the cytology of the supraoptic nucleus of the rat, has also arrived at the result that the cells show signs indicative of a strong protein synthesis mechanism (6). Furthermore, he confirmed this by showing that there is normally a strong phosphatase activity in the supraoptic and paraventricular nuclei of the rat's hypothalamus (8).

The present work constitutes an attempt to determine in what degree changes in the phosphatase activity of the hypothalamic nuclei may be observable in connection with dehydration and rehydration, as compared with the simultaneously occurring neurosecretory activity.

Aided by a grant from the Sigrid Jusélius Foundation.

MATERIAL AND METHODS

The experimental animals were adult white female and male rats, altogether 40 animals. The method of procedure was as follows: 20 animals were deprived of water and given only dry food during eight days, four of them being exterminated after this time. The remaining 16 animals received then water *ad libitum* and were killed in groups of four after a period of one day, three days, seven days and 12 days, respectively, since the termination of the dehydration period. The controls were given similar dry food as the animals of the test series but they received water *ad libitum*, in addition to this throughout the time of the tests. Four control animals were also killed at the same time with each of the test animal groups. All animals were exterminated by rapid decapitation, and the hypothalamus was immediately excised from the controls as well as the animals of the test series. Half of the tissue samples from each group of animals killed during the tests were fixed for one day in Bouin's solution and treated in the customary manner; the cuts prepared from them were stained by means of Gomori's aldehyde-fuchsin method (4). The other half of the tissue samples taken from each group was fixed for two hours in neutral 4 per cent formalin and the acid phosphatase activity was demonstrated by Gomori's method in the modification presented by Eränkö (7).

RESULTS AND CONCLUSIONS

Neurosecretion. — It is found on study of the magnocellular nuclei of the hypothalamus by means of the aldehyde-fuchsin method that eight days' dehydration produces hypertrophy of the cells and a distinct decrease of the neurosecretory substance in them. After the first day of rehydration the quantity of neurosecretory material in the cells is already slightly increased, and after the seventh day of rehydration a quantity of aldehyde-fuchsin-positive granules slightly in excess of normal is observable in the cells. After the twelfth day of rehydration, however, the aldehyde-fuchsin-positive reaction in the cells is similar to that in the cells of the control series. This result is in agreement with previous investigations although we were not able to observe such a strong stage of

over-compensation in the formation of neurosecretory material as has been described by Bachrach (10, 5, 13, 1). However, our experimental animals were not killed until after the seventh day of rehydration, whereas the corresponding observations of Bachrach refer to animals that had been rehydrated for six days. Since the normalisation of the neurosecretory activity had certainly been effected within 12 days since the commencement of rehydration, it is conceivable that one day may already have produced a certain levelling of the quantity of neurosecretory substance, and the difference of the results might be attributer to this fact.

Acid Phosphatase. — In the supraoptic nucleus as well as in the paraventricular nucleus, acid phosphatase activity was observed in the control animals both in the nucleoli of the cells, in the nuclear membrane and also in the cytoplasm itself, in accordance with earlier investigations (8, 3). On comparison of the phosphatase activities of the said nuclei of the animals in the control and test series after an incubation time of one hour as well as of two hours it was found that the animals dehydrated during eight days showed a distinct increase of activity as compared with the controls (Fig. 1, 2, 3 and 4). On the other hand no differences in acid phosphatase activity were observed between the test animals and the controls during the rehydration period.

Our results are in agreement with the investigations according to which a strong protein synthesis takes place in the cells of the magnocellular hypothalamic nuclei (*e.g.* 13, 6, 8). The enzyme activity demonstrable by means of acid phosphatase was remarkably increased upon dehydration, probably as a result of strong stimulation of the cells. A similar result is indicated by the investigation of Pepler & Pearse according to which the histochemically demonstrable specific acetylcholinesterase activity is remarkably increased when the cells of the said hypothalamic nuclei are stimulated, in this particular case with hypertonic NaCl solution (11). The return to normal seems to take place rapidly since even after the first day no significant difference could be observed any more between the test animals and the controls. It seems that the changes observed in the neurosecretory substance on one hand and the simultaneous changes of acid phosphatase on the other hand are not concurrent during the rehydration phase; this appears plausible indeed since the stainable neurosecretory substance primarily

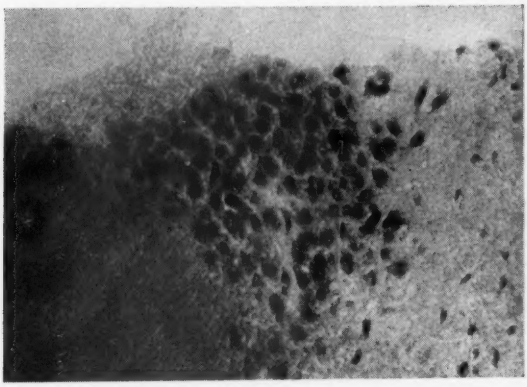


Fig. 1. — A normal acid phosphatase activity in the supra-optic nucleus of the control rat. × 120.

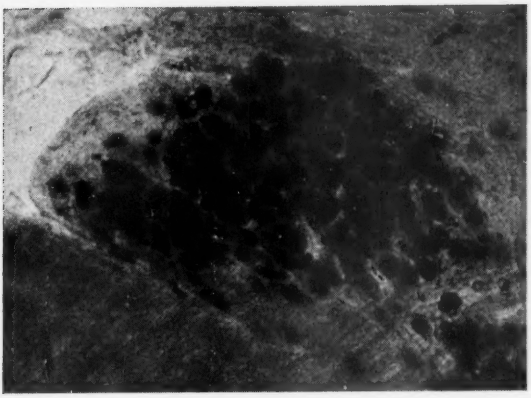


Fig. 2. — A strong acid phosphatase activity in the supra-optic nucleus of the dehydrated rat. × 120.

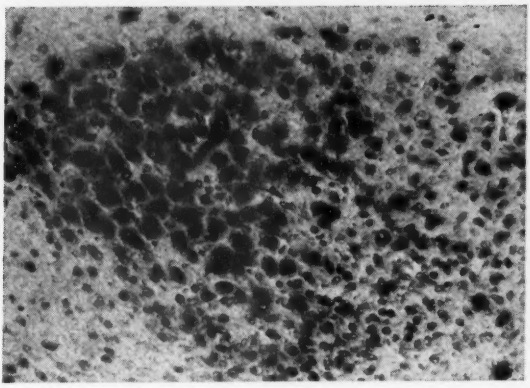


Fig. 3. — A normal acid phosphatase activity in the para-ventricular nucleus of the control rat. × 120.

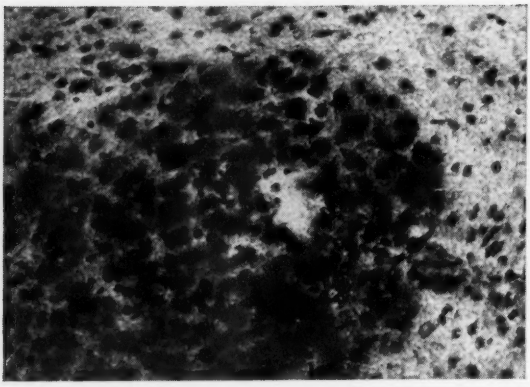


Fig. 4. — A strong acid phosphatase activity in the paraventricular nucleus of the dehydrated rat. × 120.

reflects the product of the cells in the nuclei, whereas the acid phosphatase gives an indication of the activity of these cells. The more abundant occurrence of neurosecretory substance at about the middle of the rehydration period than normally is obviously due to decreased peripheral need of antidiuretic hormone since, according to our results, the degree of activity of the cells appears to be normal.

SUMMARY

With rats as experimental animals, the effect of dehydration and rehydration simultaneously upon the acid phosphatase and the neurosecretory substance of the magnocellular hypothalamic nuclei has been studied. After eight days' dehydration a strong increase of acid phosphatase activity was observed in the cells, whereas this activity was normal again already after the first day of rehydration, This result was compared to the changes in the quantity of neurosecretory substance manifesting themselves at the same time.

REFERENCES

1. Bachrach, D.: Z. Zellforsch. 1957:*46:*457.
2. Bachrach, D.: Z. Zellforsch. 1957:*47:*147.
3. Cohn, P., and Richter, D.: J. Neurochem. 1956:*1:*166.
4. Dawson, A. B.: Anat. Rec. 1953:*115:*63.

5. EICHNER, D.: Z. Zellforsch. 1954:*40:*151.
6. ERÄNKÖ, O.: Ann. med. exper. et biol. Fenn. 1951:*29:*158.
7. ERÄNKÖ, O.: Ann. med. exper. et biol. Fenn. 1951:*29:*287.
8. ERÄNKÖ, O.: Acta Physiol. Scandinav. 1951:*24:*1.
9. KOVÁCS, K.: Naturwissenschaften. 1956:*43:*425.
10. ORTMAN, R.: Z. Zellforsch. 1951:*36:*92.
11. PEPLER, W. J., and PEARSE, A. G. E.: J. Neurochem. 1957:*1:*193.
12. SCHARRER, E., and SCHARRER, B.: Recent Progress in Hormone Research 1954:*10:*183.
13. SCHARRER, E., and SCHARRER, B.: Handbuch der mikroskopischen Anatomie des Menschen. 1954:*Bd. VI/5:*953.

FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

USE OF PHENOLPHTHALEIN PHOSPHATE-CHROMATE PLATE AS A SELECTIVE ISOLATION MEDIUM FOR MICROCOCCI

by

TIMO KOSUNEN and M. E. PARMALA

(Received for publication May 10, 1958)

In a previous report (1) we showed the possible importance of the phenolphthalein phosphate-chromate (PPC) plate in diagnosing the micrococci. The phosphatase test on the PPC plate was compared with the usual coagulase and kinase tests. — In this paper some results are described, which were obtained with the PPC plate as a selective primary plate in isolating the micrococci from samples containing Proteus and Pseudomonas species. These bacilli, by their swarming or by their toxic metabolic products, often make the detection of any other bacteria in the same sample difficult. *

MATERIAL AND RESULTS

The analysed material consisted of 22,138 routine samples sent to our Institute during the years 1956—57. These samples were cultivated on blood, McLeod, Drigalski and PPC plates. The results were compared with the analyses of 17,730 analogous routine samples from the years 1953—54. In these, otherwise identical control analyses the PPC plate was not used. Table 1 shows that the two groups of samples are fairly comparable.

* The added chromate ion makes the medium selective for micrococci; the phenolphthalein phosphate makes the differentiating of Micrococcus pyogenes and Micrococcus saprophyticus very easy.

TABLE 1

THE NUMBER OF PROTEUS MIRABILIS, PROTEUS VULGARIS, PSEUDOMONAS AERUGINOSA AND MICROCOCCI ISOLATED DURING THE YEARS 1953—54 AND 1956—57.

Bacteria	1953—54 Number of Samples 17,730		1956—57 Number of Samples 22,138	
	Number of Strains Isolated	Per Cent of All Samples	Number of Strains Isolated	Per Cent of All Samples
Proteus mirabilis..	342	1.93	633	2.86
Proteus vulgaris ..	118	0.67	111	0.50
Pseudomonas aeruginosa	615	3.47	1074	4.85
Micrococus	3153	17.78	3257	14.71

Table 2 gives the observed number of samples containing micrococci with Proteus mirabilis, Proteus vulgaris, or Pseudomonas aeruginosa. This table also shows the ratio between samples containing the mixed floras and samples containing pure cultures of

TABLE 2

NUMBER OF SAMPLES CONTAINING MICROCOCCI WITH PROTEUS MIRABILIS, PROTEUS VULGARIS OR PSEUDOMONAS AERUGINOSA

Mixed Flora	1953—54		1956—57	
	Number of Samples Containing Mixed Floras	Per Cent of All Samples Containing the Corresponding Gram-negative Rod	Number of Samples Containing Mixed Floras	Per Cent of All Samples Containing the Corresponding Gram-negative Rod
Proteus mirabilis + Micrococcus	19	5.5	193	30.5
Proteus vulgaris + Micrococcus	15	12.7	43	38.7
Pseudomonas aeruginosa + Micrococcus	145	23.6	446	41.5
Total	179		682	

TABLE 3

COMPARISON OF PHOSPHATASE AND COAGULASE TESTS IN DIFFERENTIATING THE MICROCOCCI

	Phosphatase Test	Coagulase Test	Number of Samples	Per Cent of All Tested Samples
Identical results ..	+	+	2737	92.2
	—	—	267	
Different results ..	—	+	141	7.8
	+	—	112	
Total			3257	100.0

the corresponding gram-negative rods. As a whole, during the time PPC plate was used, the micrococci were found in samples containing these rods three times more often in 1956—57 than in the corresponding samples in 1953—54. In samples containing Proteus mirabilis the micrococci were found nearly six times more often in 1956—57 than in 1953—54.

The phosphatase and coagulase tests were also performed to the micrococci isolated in 1956—57 (Table 3) Identical results in both tests were obtained in 92.2 per cent of the tested micrococci. This percentage agrees well with the results (91 per cent) of our previous report (1). The phosphatase test was carried out as described before (1). For coagulase test the micrococci were inoculated from plate cultures in tubes containing 1 ml of 1/10 saline dilution of human plasma. The results were read after 20 hours' incubation at 37°C.

DISCUSSION AND CONCLUSIONS

The characteristical swarming properties of Proteus mirabilis and Proteus vulgaris often make the detection and isolation of other bacteria from the same samples difficult. The results presented here rather clearly show the usefulness of the PPC plate when detecting micrococci from samples containing any of these two Proteus species. The advantage of the PPC plate was not so apparent when detecting micrococci from samples containing Pseudomonas aeruginosa. The metabolic products of Pseudomonas aeruginosa can also inhibit the growth of other bacteria. Its

swarming properties are less marked than those of Proteus mirabilis and Proteus vulgaris. Therefore, the detection of accompanying micrococci by the usual methods is easier in Pseudomonas samples than in those containing the Proteus species. The increase in mixed flora is rather large, according to our series, and it seems reasonable to presume it to be caused mostly by the selective culture method used. It is well known that the use of antibiotics has increased the number of infections where Proteus and Pseudomonas species are secondary invaders, Perhaps this could be partly responsible for the observed increase in frequence of the mixed floras.

Due to the effective selectivity of the PPC plate, even relatively small amounts of micrococci can be detected. Because it is possible to differentiate between Micrococcus pyogenes and Micrococcus saprophyticus directly with the PPC plate, a lot of time is also saved by its use. It is recommendable to use the PPC plate as a primary culture medium in cases where the micrococci are supposed to grow as a mixed flora, especially together with Proteus and Pseudomonas species.

SUMMARY

When phenolphthalein phosphate-chromate plate was used as a primary culture medium the isolation of micrococci from samples containing Proteus mirabilis could be performed nearly six times, from samples containing Proteus vulgaris three times, and from samples containing Pseudomonas aeruginosa nearly two times more often than before.

Of the over 3000 tested micrococci 92.2 per cent gave identical results in the coagulase and phosphatase tests.

REFERENCE

1. Parmala, M. E., and Kosunen, T.: Ann. Med. Exp. Biol. Fenniae 1956:*34*:154.

FROM THE RESEARCH LABORATORIES OF THE STATE ALCOHOL MONOPOLY
HELSINKI, AND THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY,
UNIVERSITY OF HELSINKI

EFFECT OF ETHYL ALCOHOL ON THE SUSCEPTIBILITY OF MICE TO STAPHYLOCOCCAL INFECTION

I

by

O. WASZ-HÖCKERT, TIMO KOSUNEN, and J. KOHONEN

(Received for publication May 10, 1958)

This investigation, a link in a long-term study program concerning the effect of various factors on the host-parasite relationship, deals with the question of whether the use of ethyl alcohol affects the resistance of the host organism to infections, a point of clinical and social interest.

The review by Pickrell (5) showed that the effect of alcohol intoxication on infection and immunity has been a subject of experimental study since back in 1885 when Koch (2) found that guinea pigs, when given alcohol and inoculated with cholera, were more susceptible to cholera than the control animals without alcohol. The problem was also studied in Finland some fifty years ago by Laitinen and Oker-Blom (3, 4).

Although it is generally agreed that the survival time is shorter for infected, alcohol intoxicated animals than for non-intoxicated ones, no sufficient explanation has been given of the probable resistance-lowering effect of alcohol.

The problem is still worth attention. The use of a method by which it is possible to measure quantitatively the degree of infection seemed to be indicated.

Aided by grants from the Finnish Foundation for Alcohol Studies and State Commission for Sciences.

In approach to the problem the present authors used a staphylococcal infection and a mice strain the host-parasite balance of which is well known from previous experiments (7, 8).

MATERIAL AND METHODS

Mice. — One hundred and thirty-five Swiss Albino Webster mice, ranging in weight from 19.5 to 25 g, were used.

Bacterial Culture. — *Staphylococcus aureus*, strain 'Orion', cultured for 24 hours in broth, was used in an 1: 2 dilution. The challenge dose was 0.2 ml intravenously in one of the tail veins.

Ethanol. — Eight per cent w/w ethanol-water was used.

Technique. — The technique employed involved noting the survival time and making a bacterial enumeration. The quantitative method of bacterial enumeration is described in detail earlier (6, 7).

PLAN OF THE EXPERIMENT

The mice were kept in wire mesh bottom cages, 15 mice to a cage, in an air conditioned room. Food pellets of a standardised composition were available *ad libitum.*

120 mice were challenged. They were divided into two equal groups and treated as follows:

Ethanol Group. — In each cage an inverted bottle of drinking fluid containing an aqueous solution of ethanol was available for the mice.

Control Group. — The fluid intake in these cages was arrenged in the same way as for the former group except that the bottles contained water only.

15 mice were treated as follows:

A Second Control Group. — These mice were not challenged, but were given ethanol as in group 1.

Mice mortality records were kept and the survival time calculated. In groups 1 and 2, one mouse from each cage was withdrawn by random sampling. Thus, 8 mice a day were taken during the first five days, then three times weekly. The mice were sacrificed, the spleen, liver and kidneys removed by sterile technique, homogenized and further investigated by the bacterial enumeration technique mentioned above.

RESULTS

Ethanol in given doses did not alter the behavior of the mice, nor were there any detectable amounts of ethanol in their blood, determined by the Widmark method. No differences were observed in the increase of weight in the three groups.

TABLE 1

Group	Number of Mice	Cumulative Number of Deaths							
		2	4	6	8	10	12	14	16 Days
Ethanol group.....	60	0	0	2	5	6	8	11	13
Control group	60	0	0	1	1	1	1	1	1

Survival. — The mice of the ethanol group were thus susceptible to staphylococci: 13 out of 60 mice died in comparison with one out of 60 in the control group (Table 1). The difference between the groups is highly significant ($P < 0.005$).

A second control group of 15 mice was kept in the same conditions as the ethanol group, except for the staphylococcal infection. Not one of them died during 6 weeks.

Bacterial Enumeration. — The number of bacteria in the organs (spleen, kidneys and liver) was determined during the experiment. The results are shown in Figs. 1—3.

The bacterial population curves do not show any statistically significant differences between the ethanol and non-ethanol groups in either spleens or kidneys. In the spleens the infection seemed to be washed out relatively rapidly. In the kidneys, however, the staphylococcal infection was persistent and within 3—5 days foci were seen macroscopically.

The determinations from the livers, on the other hand, showed that the number of staphylococci in the mice group given ethanol was significantly higher than in the animals which did not receive ethanol. As Fig. 3 shows, the bacterial population curve of the control mice declined in a very similar way to the curve illustrating the process in the spleen. In the ethanol group the staphylococcal population in the liver declined during the first 4 days in the same way as in the control group. From the 4th day on to the end of the experiment, the staphylococcal population in the ethanol group was

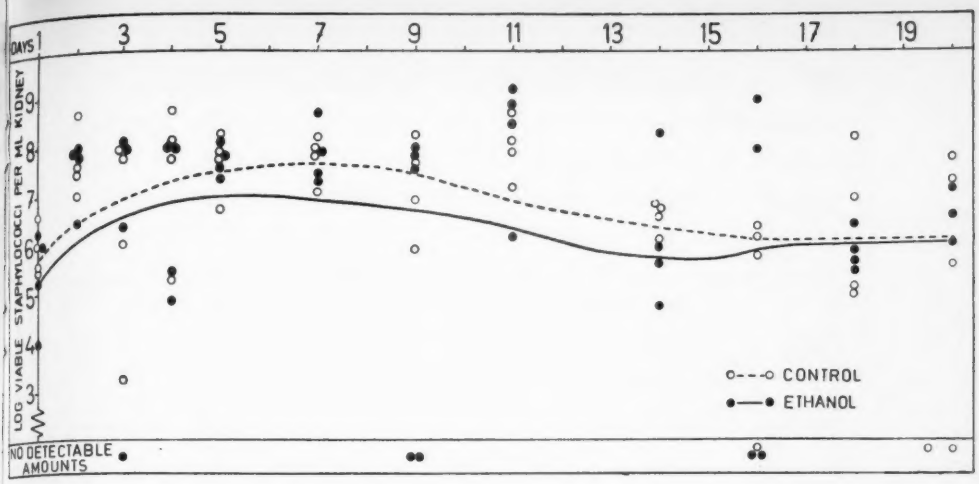


Fig. 1.

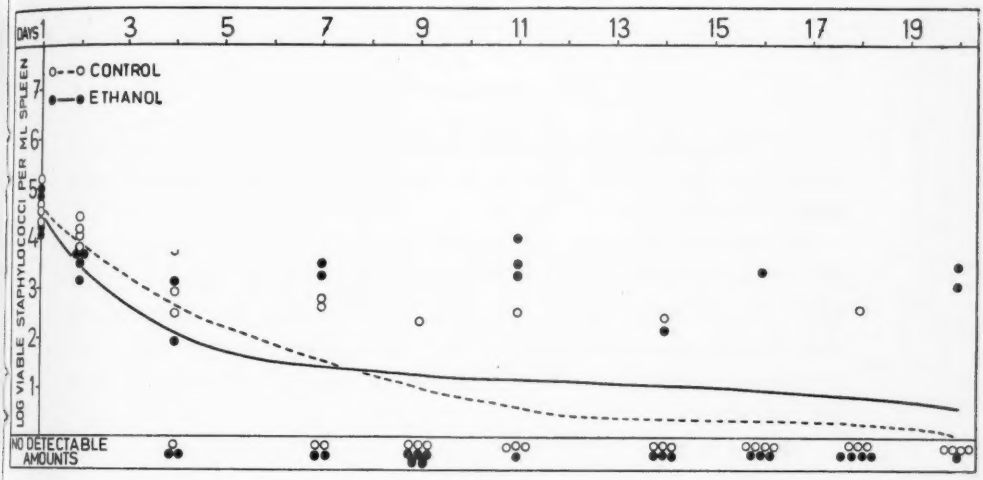


Fig. 2.

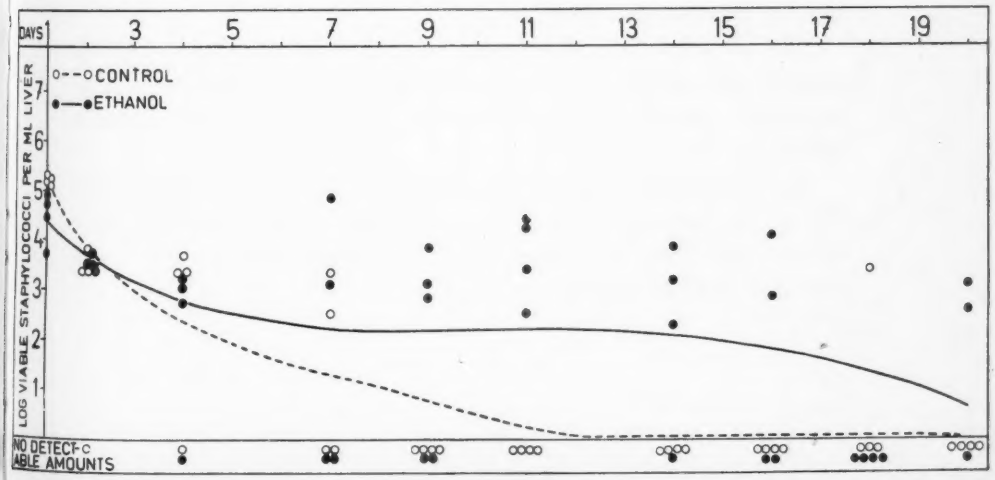


Fig. 3.

considerably greater than in the control group. The difference between the ethanol group and the control group is highly significant ($P < 0.001$), as can be seen from Table 2.

TABLE 2

Days	Ethanol Group		Control Group		Degrees of Freedom	t	P <
	Log. Viable Staphylococci per ml Liver (Mean)	n	Log. Viable Staphylococci per ml Liver (Mean)	n			
1— 4	3.44	12	3.49	12	22		—
5—20	1.94	28	0.33	28	54	4.128	0.001
1—20	2.39	40	1.28	40	78	2.413	0.025

DISCUSSION

The investigations reported in the literature generally concern ethanol intoxication in rabbits in relation to various bacterial and viral infections.

In this investigation the authors dealt with the continuous use of ethanol in a dosage which did not affect the behaviour of the mice. The investigation of Arvola *et al.* concerning the behavior of rats (1) and mice (personal communication) given various amounts of ethanol confirm our observations of the clinical effects of ethanol on this ethanol level.

We have found that the mouse is a very useful experimental animal for this kind of study. The staphylococcal infection in mice measured by the bacterial enumeration technique seems to be suitable for these experiments.

The survival time difference between infected mice receiving ethanol and control animals is evident from the present study. Analogous results have frequently been reported for various infections.

An observation of interest, however, is that the bacterial population in the livers of ethanol-consuming mice is significantly higher than in the livers of control mice. This observation indicates the need for further studies.

SUMMARY

Eight per cent ethanol as drinking fluid curtailed significantly the survival time of mice infected intravenously with staphylococci.

Using a bacterial enumeration technique, the authors were able to prove that the number of viable staphylococci in the liver of ethanol consuming mice was significantly higher than the value for the control group. The staphylococcal population in the kidneys and spleen grew similarly in both the alcohol and the control groups.

REFERENCES

1. Arvola, A., Sammalisto, L., and Wallgren, H.: Quart. J. Stud. Alc., in press.
2. Koch, R.: Gesammelte Werke, Band II; Teil 1: 69, G. Thieme, Leipzig 1912.
3. Laitinen, T.: Acta Soc. Scient. Fenn. ,1902:*29*.
4. Oker-Blom, M.: Zschr. Imm., 1913:*16*:102.
5. Pickrell, K. L.: Bull. John Hopkins Hosp., 1938:*63*:238.
6. Smith, J. H., and Dubos, R. J.: J. Exp. Med., 1956:*103*:87.
7. Wasz-Höckert, O., Eriksson, A. W., and Kosunen, T.: Ann. Med. Exp. Biol. Fenniae, 1956:*34*:438.
8. Wasz-Höckert, O., Oker-Blom, N., and Kosunen, T.: Ann. Med. Exp. Biol. Fenniae 1956:*34*:428.

FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY AND THE DEPARTMENT OF VIROLOGY, UNIVERSITY OF HELSINKI

DESTRUCTION OF *M* AND *N* BLOOD GROUP RECEPTORS OF HUMAN RED CELLS BY SOME INFLUENZA VIRUSES

by

O. MÄKELÄ [1] and KARI CANTELL [2]

(Received for publication June 16, 1958)

Myxoviruses (influenza, NDV, mumps) possess an ability to agglutinate the red cells of several animal species, to destroy the receptors in them and to be eluted spontaneously, especially at the temperature of 37° C. After an elution has taken place, the following changes in the red cells have been noticed: *a*) If the treatment has been sufficient the cells are no longer agglutinable by myxoviruses (13). There are differences, however, in the ability of various viruses and strains to destroy receptors (4). *b*) The electrophoretic mobility of the red cells has been changed (11, 1). *c*) The cells have, under certain experimental conditions, become hemolysed by mumps (21) and NDV viruses (17) or they exhibit an increased osmotic fragility (21). *d*) The Rh_0 (D) blood group receptor has been destroyed or released by mumps virus (2) and the »mononucleosis receptor» of sheep erythrocytes by influenza viruses (25). *e*) The cells have become agglutinable by incomplete anti-Rh_0 (D) agglutinins (influenza virus) (6, 26), by several normal sera (4) as well as by some plant agglutinins which do not agglutinate untreated cells (20). *f*) The cells have become susceptible to hemolysis by fresh guinea-pig serum in dilutions up to 1 to 10 (26). As far as the present writers are aware, virus treatment has not been

[1] Aided by a grant from Sigrid Jusélius Foundation.
[2] Aided by a grant from Emil Aaltonen Foundation.

found to cause other changes in red cells. Thus their ability to absorb isoagglutinins remains unaffected (23). We do not know of any studies having been made in the treatment's effect on other blood group receptors.

On the other hand, it is known that periodate treatment easily makes red cells inagglutinable by myxoviruses (12) as well as by anti-M, anti-N and anti-Rh_0 (D) (22).

In view of these observations the effect of virus treatment on the M and N was examined in this work. Since the chemical structure of both the blood group substances and the substrate of virus enzymes in particular are being actively studied, it would profit each if correlations could be found between the two fields.

MATERIAL AND METHODS

Virus strains: [1] Influenza PR8
Influenza A/Finland/1/53
Influenza B Lee

Immune Sera. — Three rabbit immune anti-M and three anti-N sera, one human immune anti-A and one human immune anti-B were used. The rabbit sera were diluted with 30 to 180 fold amounts of saline followed by absorption with human red cells (*e.g.* the anti-M with A_1N cells) so as to eliminate unspecific antibodies. Standard reagents so obtained were solely used in the work. Anti-A and anti-B were diluted before use with a ninefold amount of saline.

Preparation of Virus Allantoic Fluids. — 0.2 ml of virus allantoic fluid diluted 10^{-3} to 10^{-5} in tryptose phosphate broth and containing 500 units of penicillin and 500 μgs of streptomycin per ml was inoculated into the allantoic sac of embryonated eggs which had been previously incubated for 10—12 days at 37° C. The eggs were further incubated for 48 hours at 35° C and chilled overnight at + 4° C. The allantoic fluids were harvested, pooled and, after some hours storing at + 4° C, cleared by centrifuging for 15 minutes at 2500 r.p.m. at 0° C in a refrigerated centrifuge. The supernatant served as the virus allantoic fluid used in the experiments.

Preparation of Control Allantoic Fluids. — A technique identical with that above was used in the preparation of the control allantoic fluid except that instead of diluted virus allantoic fluid the diluent, tryptose phosphate broth, was inoculated into the eggs.

Treatment of Human Red Cells with Viruses. — Packed, washed human red cells were suspended in virus allantoic fluid with a hemagglutination titre of 1/2048 (determined with Salk's pattern method (23a) using 0.5%

[1] All strains were obtained through the courtesy of Dr. Kari Penttinen, State Serum Institute.

chicken cells) and in control allantoic fluids to give a 0.75% and 2% suspension respectively. Appropriate amounts of penicillin and streptomycin were added to give a final concentration of 500 units and 500 µgs per ml. The cell suspensions were kept at 37° C for 22 hours during which time the flasks were shaken several times. The cells were washed three times with saline and used as an approx. 3% suspension in agglutination experiments.

Agglutination and Absorption Experiments. — These were made at room temperature (19—21° C) if not stated otherwise. Each amount of serum was absorbed only once with varying cells/serum proportions. Titrations of the sera were made in bulk in saline before distributing 0.05 ml volumes to the agglutination tubes with a Pasteur pipette. Equal amounts of 3% red cell suspensions were added and the results were read after approx. two hours with the naked eye. The denominators of the final dilutions causing a clear agglutination are given in the following as titres of the sera.

RESULTS

Agglutination of Virus-treated Red Cells. — The results are in Table I and Fig. 1. Red cells treated with normal allantoic fluid behaved quite like usual test cells. The virus treated cells were agglutinated by most human AB sera the titre varying between 1 and 32. They were not markedly agglutinated in saline.

TABLE 1

THE EFFECT OF VIRUS TREATMENT OF RED CELLS ON THEIR AGGLUTINABILITY BY RABBIT ANTI-M AND ANTI-N SERA

Interaction	Serum	Red Cells Treated with						
		Normal Allantoic Fluid		PR8		AF/53		Lee
Anti-M v. M cells	M_{504}	4	8	1	1	2	4	1
	M_{315}	16	16	4	4	2	4	1
	M_{24}	16	16	1	1	1	1	1
Anti-M v. N cells	M_{504}	—	—	—	—	—	—	—
	M_{315}	—	—	2	4	2	2	1
	M_{24}	—	—	1	1	1	1	1
Anti-N v. N cells	N_{585}	8	8	4	4	4	1	
	N_{813}	8	4	1	2	1	1	
	N_{501}	8	4	8	4	4	2	
Anti-N v. M cells	N_{585}	1?	—	2	1	—	8	
	N_{813}	—	—	2	2	1	2	
	N_{501}	—	—	4	4	4	8	
Anti-A v. A_1 cells		16	16	16	16	16	16	
Anti-B v. A_1 cells		—	—	—	—	—	—	

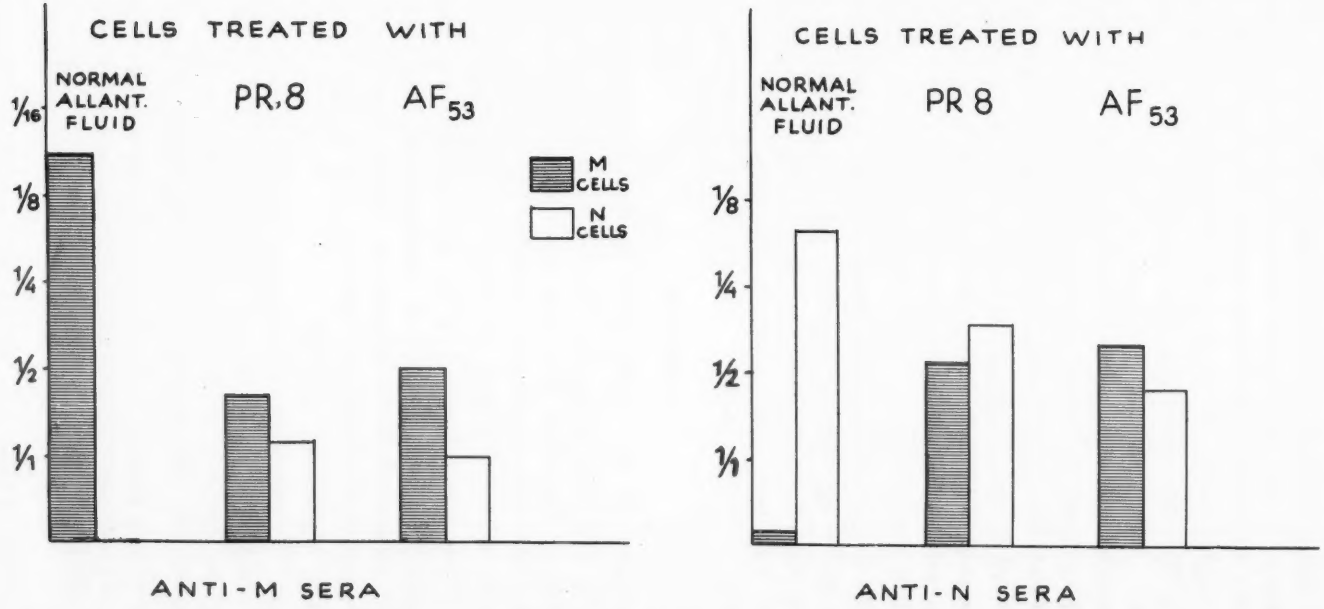


Fig. 1. — The effect of virus treatment of red cells on their agglutinability by rabbit anti-M and anti-M sera. The illustration summarizes Table 1. Each column represents the average titre from six experiments, *e.g.* the first column from testing of two M cell samples with three anti-M sera.

Anti-M sera agglutinated the virus treated cells weakly almost without respect to the MN groups. The average titre against the M cells was a little higher than that against N cells, though. The unspecific agglutination of virus treated cells was stronger by anti-N sera than by anti-M sera. No clear N specificity could be established.

The reactions with anti-A and anti-B sera of the virus treated cells and the control cells were very much alike.

Experiments like those described above were also made at the temperature of 37° C. The results being more difficult to interpret because the control cells showed considerably weaker agglutination by the sera were similar to the results above.

Absorption Experiments. — The results are given in Fig. 2. Cells treated with the Lee virus were found to absorb human immune anti-A and anti-B agglutinins almost as readily as the control cells. Anti-M and anti-N, on the contrary, do not seem to be absorbed by them at all while these agglutinins are easily absorbed by the control cells.

DISCUSSION

Interpretation of the results given in Table 1 and Fig. 1. is hampered by the fact that virus treated cells are agglutinated unspecifically by the rabbit sera used. Earlier studies have shown red cells treated with proteolytic enzymes (29, 24, 16) or with diphphteroid »T-bacteria» (16) to behave in like manner. This phenomenon has been accounted for by assuming that the reagents used contain some residual traces of unspecific antibodies despite attempts to remove them by absorption. These residua are not enough to agglutinate intact red cells but they suffice to agglutinate enzyme treated red cells (29).

Because of the unspecific agglutination it is the author's opinion that absorption experiments give a more reliable answer to the question of the existence of blood group receptors in virus treated cells. On the basis of them it seems obvious that M and N receptors, for the significant part at least, have been destroyed or released while A and B receptors have remained intact. The resistence of A and B receptors toward s virus treatment has been demonstrated earlier (23).

In our studies all influenza virus strains proved equal destroyers

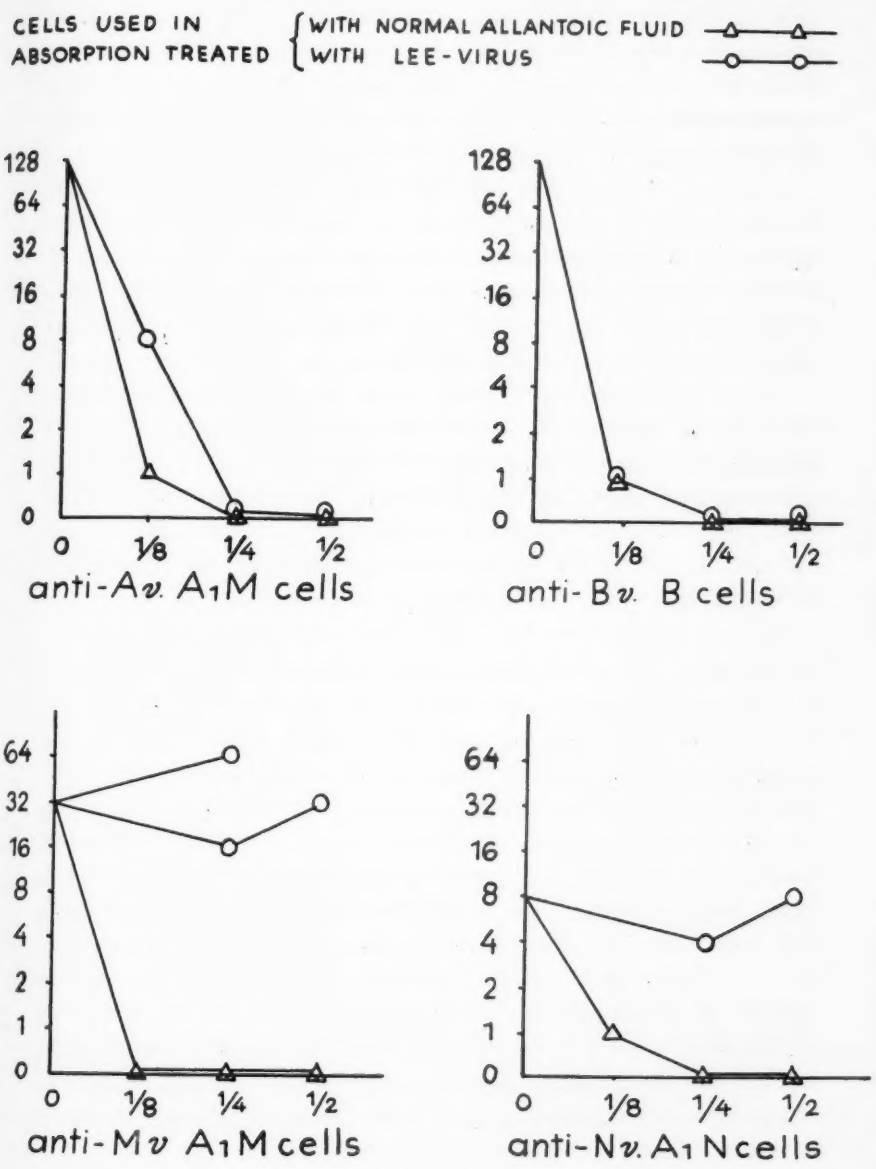


Fig. 2. — The ability of red cells treated with virus or normal allantoic fluid to absorb anti-A, anti-B, anti-M or anti-N agglutinins. Figures on the ordinate denote titre of serum against untreated cells before and after absorptions. Those on the abscissa indicate red cells-to-serum ratio chosen for each absorption.

of M and N receptors. This did not come as a surprise since these strains obviously all affect the same receptor and for the detection of any quantitative differences the adopted virus treatment technique was too efficient because each strain was capable of making the cells unagglutinable by other strains.

Comparative studies on the ability of different myxoviruses to destroy the M and N receptors of red cells could perhaps throw light on the interrelationships of the myxoviruses. This new method of measuring the virus receptors of red cells might, in particular, yield further information about a different receptor possibly employed by some myxoviruses (27, 28).

There is not much knowledge about the structure of M and N blood group substances. It has been demonstrated that a polysaccharide fraction isolated from the stroma of red cells inhibits corresponding agglutinins specifically (14). Judging by this it would seem that the M and N blood group substances are polysaccharides; but the structure of the »receptors», *i.e.* terminal groups responsible for the specifity, is unsolved as far as the authors know.

One of the reasons why this study was undertaken was the considerable amount of data recently obtained of the substrate of the influenza virus enzyme responsible for receptors destruction of red cells. A view which is gaining ground is that sialic acid, the group name for all acetylated neuraminic acids, has a key position in the structure of the substrate. It has been shown that: a) Virus enzyme splits off a chromogenic substance probably sialic acid from human erythrocyte stromata (15); and the isodynamic receptor destroying enzyme of *Vibrio cholerae* cleaves N-acetylneuraminic acid from stromata. This has been isolated in crystalline form (19). b) Virus splits off the sialic acid unit from some soluble mucins capable of inhibiting the viral hemagglutination (7, 18), and from neuramin-lactose, a compound of known structure. The linkage cleft in neuramin-lactose is of the 0-glycosidic type (9). c) neuraminic acid in combination with a trace of galactosamine, possess inhibitory action on virus hemagglutination (3).

On basis of the above data the virus enzyme has been defined as sialidase or neuraminidase cleaving the ketosidic linkage joining the acetylated neuraminic acid to another sugar or sugar derivative. Gottschalk even holds the opinion that the sialic acid unit cleft takes a terminal position (10).

Neuraminidase is the only known influenza virus enzyme (8). If splitting off of terminal sialic acid units is the only change caused by the viruses it should be possible to conclude, on the basis of the above results, that sialic acid is an essential part of the M and N receptors. This suggestion is perhaps supported by the observation that N-acetylneuraminic acid[1] inhibits rabbit anti-M and anti-N agglutinins though weakly (5).

The supposed relationship between the viral and MN receptors is compatible with the fact that both are easily destroyed by the periodate treatment (12, 22). On the other hand, red cells whose MN receptors have been destroyed by papain are agglutinated by viruses as normal cells (5).

Some light could perhaps be thrown to the relations between viral and blood group receptors by investigating digests prepared from erythrocytes by enzymes or by viruses. This work is under preparation.

SUMMARY

It has been found that after treatment with influenza viruses (PR8, A/Finland/53, Lee) human red cells of blood groups M and N are not agglutinated specifically by their homologous rabbit antisera, nor are they capable of absorbing these antibodies. The treated A and B cells are agglutinated by human immune anti-A and anti-B like the control cells and they absorb anti-A and anti-B nearly as readily as the control cells do. The implications of these findings with regard to the study of the structure of the M and N receptors is discussed.

Note Added in Proof. — After the submission of this paper the writers became aware of the work by G. F. Springer and N. J. Ansell in Proc nat. Acad. Sci. 1958:*44:*182 in which it was reported, that certain influenza viruses and R.D.E. of *Vibrio cholerae* are capable of inactivating or removing agglutinogens M and N.

---

[1] The sample of N-acetylneuraminic acid was kindly provided by Dr. Lars Svennerholm, Medicinisk-Kemiska Institutionen, Göteborgs Universitet, Göteborg, Sweden.

REFERENCES

1. Ada, G. L., and Stone, J. D.: Nature 1950:*165:*189.
2. Bigley, N. J., Chandler, R. W., and Dodd, M. C.: J. Immunol. 1958:*80:*85.
3. Bogoch, S.: Virology 1957:*4:*458.
4. Burnet, F. M., McCrea, J. F., and Stone, J. D.: Brit. J. exp. Path. 1946:*27:*228.
5. Cantell, K., and Mäkelä, O.: Unpublished observations.
6. Chu, C. M., and Coombs, R. R. A.: Lancet 1947:*1:*484.
7. Gottschalk, A.: Yale J. Biol. Med. 1954:*26:*352.
8. Gottschalk, A.: Physiol. Rews. 1957:*37:*66.
9. Gottschalk, A.: Biochem. Biophys. Acta 1957:*23* 645.
10. Gottschalk, A.: Nature 1958:*181:*377.
11. Hanig, M.: Proc. Soc. exp. Biol. Med. 1948:*68:*385.
12. Hirst, G. K.: J. exp. Med. 1948:*87:*301.
13. Hirst, G. K.: J. exp. Med. 1950:*91:*161.
14. Hohorst, H. J.: Zschr. Hyg. 1954:*139:*561.
15. Howe, C., Rose, H. M., and Schneider, L.: Proc. Soc. exp. Biol. Med. 1957:*96:*88.
16. Hummel, K.: Zbl. Bakt., I Abt. Ref. 1955:*155:*1.
17. Kilham, L.: Proc. Soc. exp. Biol. Med. 1949:*71:*63.
18. Klenk, E., Faillard, H., and Lempfrid, H.: Zschr. Physiol. Chem. 1955:*301:*235.
19. Klenk, E., and Lempfrid, H.: Ibid. 1957:*307:*278.
20. Krüpe, M., and Dötzer, W.: Ann. Med. exp. Biol. Fenn. 1957:*35:*1.
21. Morgan, H. R., Enders, J. F., and Wagley, P. F.: J. exp. Med. 1948:*88:*503.
22. Morgan, W. T. J., and Watkins, W. M.: Brit. J. exp. Path. 1951:*32:*34.
23. Overman, J. R., and Friedewald, W. F.: J. Immunol. 1949:*62:*415.
24. Rosenfield, R. E., and Vogel, P.: Transact. N. Y. Acad. Sci. Series 2 1951:*13:*213.

24a. Salk, J. E.: J. Immunol. 1944:*49:*89.

25. Springer, C. F., and Rapaport, M. J.: Proc. Soc. exp. Biol. Med. 1957:*96:*103.
26. Stone, J. D.: Austral. J. exp. Biol. 1947:*25:*137.
27. Stone, J. D., and Ada, G. L.: Brit. J. exp. Pat. 1952:*33:*428.
28. White, J.: Ibid. 1953:*34:*668.
29. Wiener, A. S., and Katz, L.: J. Immunol. 1951:*66:*51.

FROM THE DEPARTMENT OF ANATOMY, VETERINARY COLLEGE OF HELSINKI, AND THE DEPARTMENT OF ANATOMY, UNIVERSITY OF TURKU

ALDEHYDE-FUCHSIN-POSITIVE GRANULES IN THE PURKINJE CELLS OF SOME LABORATORY ANIMALS

by

S. TALANTI and E. KIVALO[1]

(Received for publication May 29, 1958)

As far as we know, the chemical character of the aldehyde-fuchsin-positive substance has not been clarified, and quite probably this response is not even specific to any one homogeneous substance. This is already indicated by the fact that the said substance is encountered in tissues of so greatly different character (5). The occurence of aldehyde-fuchsin-positive substance in the cells of the central nervous system of vertebrates has been studied very little only. Most abundantly this substance is found in the hypothalamo-neurohypophysial neurosecretory system. Its distribution there is the same as that of the so-called Gomori substance (2, 7). The substance is encountered in the cells of the supraoptic nucleus as well as the paraventricular nucleus and in the tracts leading from these to the neurohypophysis, as also in the cellular tissue of the neurohypophysis. Aldehyde-fuchsin-positive substance also occurs elsewhere in the region of the central nervous system, above all in some paraventricular formations, e.g., in the subcommissural organ (17). In some other nerve cells, too, such as in the cells of the nucleus of the third cerebral nerve, aldehydefuchsin-positive substance is noted, although it differs from the previously mentioned occurrences in regard to its intensity of staining (14).

[1] This study was supported by grant from the State Commision of Natural Sciences.

In the Purkinje cells of the cerebellum, aldehyde-fuchsin-positive substance has previously been observed in man (13). The purpose of the present investigation was to study the presence and distribution of the said substance in the Purkinje cells of some laboratory animals and, at the same time, to compare it with the PAS-positive substance.

MATERIAL AND METHODS

The series consisted of cerebella of the rabbit, guinea pig, hamster, rat and mouse. Only fresh material was used. It was endeavoured to avoid postmortal injuries during removal of the brain from the skull. The parts of the cerebellum were immediately immersed in Bouin's fluid and in 10% formalin solution. Fixation was performed for not less than 24 hours. Embedding in paraffin. The sections were cut sagittally, transversally and vertically at 5 to 20 micra. After deparaffinization and dehydration through the alcohol and xylene, aldehyde-fuchsin staining was performed according to Gomori's method as modified by Landing *et al.* (9).

PAS staining was carried out according to the method of McManus. Part of the specimens were stained after saliva treatment. The comparison studies between different animal species were carried out in identical conditions, at one and the same time and with the same solutions.

RESULTS

Aldehyde-fuchsin-positive substance could be demonstrated as small granules in the cytoplasm of the Purkinje cells in the cerebellum of all investigated animals. The substance occurred exclusively as granules, which could, however, form agglomerations of verying size. Granules and granule clusters could also be seen around the Purkinje cells as a narrow zone on the boundary between the molecular layer and the granular layer. The last-mentioned substance occurred particularly abundantly around the cell bodies in the fibullary network but it was also observable elsewhere in the interstices of the Purkinje cells. No particular tendency of the material to accumulate around blood vessels could be noted although it was encountered also around the vessels.

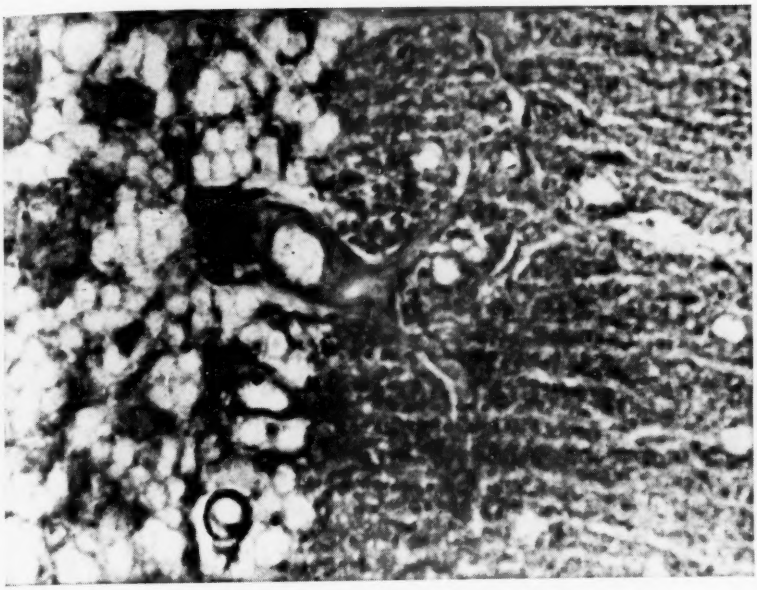


Fig. 1. — Purkinje cell of the guinea pig. Aldehyde-fuchsin-positive granules outside of the cell near the point of emergence of the dendrites. × 480.

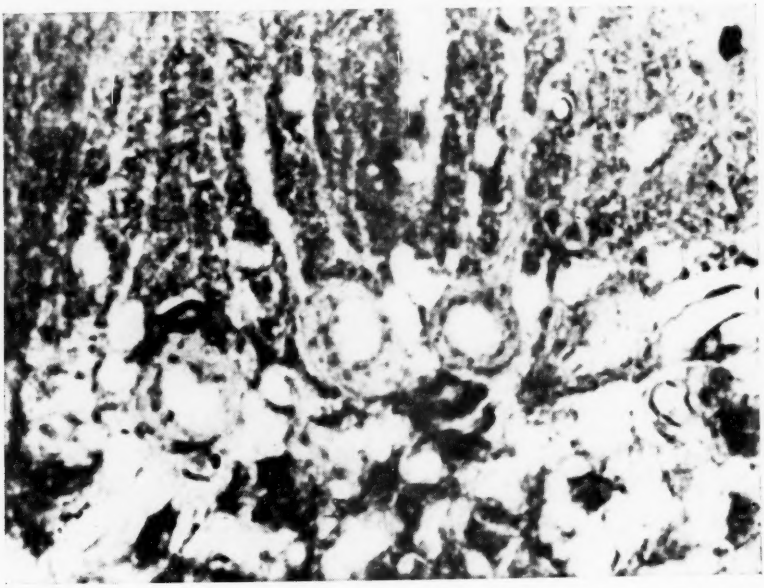


Fig. 2. — Purkinje cells of the mouse. Aldehyde-fuchsin-positive granules inside the cells. × 480.

Although the substance under consideration was established in all investigated animals, certain small variations could be noted in its distribution. The rabbit had granules mostly in the cytoplasm of the Purkinje cells, only a very small amount outside of them. With the guinea-pig, again, granules could be seen in distinctly greater quantity outside of the Purkinje cells near the point of emergence of the dendrites; the hamster had abundant granules outside the Purkinje cells and particularly between the molecular layer and the granular layer along their boundary line, although more abundantly on the side of the molecular layer. In the rat, granules were observed abundantly outside the cells and only in small quantities the cells themselves, whereas the mouse had more granules inside the cells. In all animals the granules within the cells were found to be accumulated primarily in the upper part of the cell near the cell membrane, while hardly any granules occurred in the basal part of the cells, (fig. 1 and 2).

The PAS-positive granules appeared to behave completely in accord with the aldehyde-fuchsin-positive granules, and it is therefore obvious that one and the same substance was stained by both methods.

DISCUSSION

It is evident from the result of the investigation that aldehyde-fuchsin-positive substance occurs in all animals investigated in this instance, and we are thus concerned with a biologically consistent phenomenon although the distribution of the granules among the different zones of the Purkinje cells did not seem to be fully identical in the different animals.

Nothing can be said with certaintly in regard to the character of the aldehyde-fuchsin-positive granules on the basis of this investigation. The staining method is not specific for any one definite chemical substance; on the contrary obviously substances of very variegated chemical character, such as e.g. certain proteins and mucopolysaccharides, respond positively to aldehydefuchsin owing to certain structural parts of their molecules (4, 1, 12). The method was originally developed for the staining of elastic tissue but it has later been found to be suitable (9), among other things, also for the description of the secretory functions occurring in the nerve

cells. In this respect the method can be parallelled with Gomori's chrome-alum-hematoxylin-phloxin method (2, 7, 9). The similar distribution of the PAS-positive substance in the Purkinje cells and in their neighbourhood might imply that one and the same substance is concerned. PAS-positively indicates the presence of polysaccharides and glycoproteins. Immersion in saliva does not cancel the response, which excludes the possibility that a reaction produced by polysaccharides would be concerned and rather argues in favour of a carbohydrate-protein complex (3).

Hypotheses in favour of a secretory function of the Purkinje cells have been presented before (10, 11, 16). The strong acid phosphatase activity of the Purkinje cells, too, would indicate this (8). On the other hand the aldehyde-fuchsin-positive granules now in question cannot be compared with the hypothalamic neurosecretory material because it is not PAS-positive (6). Also, the neurosecrete granules of the dog, for instance, are not similar under the phase contrast microscope to the granules of the Purkinje cells (15), nor is the distribution of the granules now in question in the Purkinje cells and in their vicinity indicative of any phenomenon corresponding to that of the neurosecretion in the hypothalamus.

SUMMARY

The aldehyde-fuchsin-positive granules occurring in the Purkinje cells of the cerebellum of the rabbit, guinea-pig, hamster, rat and mouse were investigated. They were found to exist in all investigated animals, although grouped according to distribution patterns characteristic of each species. The correlation between the occurrence of this substance and the potential neurocrine activity of the Purkinje cells has been discussed.

REFERENCES

1. Bangle, Jr., R.: J. Histochem. and Cytochem. 1954:*2*:291.
2. Dawson, A. B.: Anat. Rec. 1953:*117*:499.
3. Dixson, K. C., and Herbertson, B. M.: J. Path. and Bact. 1950:*62*: 335.
4. Graumann, W.: Acta Histochemica, 1956:*3*:226.
5. Holczinger, L.: Acta Histochemica, 1957:*5*:25.

6. Howe, A., and Pearse, A. G. E.: J. Histchem. and Cytochem. 1956: *4:*561.
7. Kivalo, E., and Talanti, S.: Acta Endocrinologica, 1957:*26:*471.
8. Kivalo, E., and Lehtinen, L.: Ann. med. exper. et biol. Fenn. 1957: *35:*387.
9. Landing, B., Hall, H., and West, C.: Lab. Investigation, 1956:*5:* 1956.
10. Mosinger, M.: C. R. Acad. Sci., 1951:*233:*982.
11. Mosinger, M.: C. R. Soc. Biol., 1956:*150:*1588.
12. Scott, H. L., and Clayton, B. P.: J. Histochem. and Cytochem 1953:*1:*336.
13. Shanklin, W. M., Issidorides, M., and Nassar, T. K.: J. Comp. Neur. 1957:*107:*315.
14. Sloper, J. C.: J. Anat. 1955:*89:*301.
15. Takahashi, S.: Arch. hist. jap. 1957:*12:*297.
16. Thomas, O. L.: J. Comp. Neur. 1951:*95:*73.
17. Wislocki, G. B., and Leduc, E. H.: J. Comp. Neur. 1954:*101:*283.

FROM THE DEPARTMENT OF ANATOMY, VETERINARY COLLEGE, HELSINKI, AND
FROM THE DEPARTMENT OF ANATOMY, UNIVERSITY OF TURKU

THE GANGLION CELLS OF THE AUTONOMIC NERVOUS SYSTEM

INVESTIGATIONS ON SOME DOMESTIC ANIMALS, WITH SPECIAL REFERENCE TO THE ALDEHYDE-FUCHSIN POSITIVE GRANULES

by

S. TALANTI and E. KIVALO [1]

(Received for publication June 24, 1958)

A large amount of evidence based on morphologic and physiologic studies (16) and apparently conclusive has been produced in support of Scharrer's neurosecretory theory. The secretory function of the nerve cells could be observed in the area of the *nucleus supraopticus* and the *nucleus paraventricularis* in the hypothalamus*n* of various species, while the neurosecretion in other parts of the nervous system is still enigmatic. Morphologic investigations have not yielded as clear results as in the above nuclei. According to a number of investigations cytoplasmic granules or vacuoles produced with various staining methods were to be found in the nerve cells of the autonomic nervous system (2, 4, 5, 6, 7, 9, 10, 13, 14, 15, 19). These formations are, as a rule, explained to be either degenerative phenomena or appearances of neurosecretion, but no actually conclusive evidence has been presented in favour of one or the other.

The histologic investigation of the secretion of the central nervous system is made according to a few staining methods applicable in demonstrating the neurosecretory material. Gomori's chrome-alum-haematoxylin-phloxin method and Gomori's aldehyde-

[1] Aided by a grant from the Natural Science Committee of the State.

fuchsin method are the most important and the most used methods. The first mentioned method is also employed in the investigation of the ganglion cells of the autonomic nervous system (4, 5). Previously these ganglion cells have not been investigated with the aldehyde-fuchsin method, according to literature accessible to us. This method has proved to be particularly applicable in demonstrating neurosecretory phenomena in the hypothalamic-hypophysial system (3, 11, 12). The purpose of this study was to investigate eventual neurosecretory phenomena of the ganglion cells of the autonomic nervous system by using the aldehyde-fuchsin method, furthermore, to demonstrate the distribution of the aldehyde-fuchsin positive material in these cells.

MATERIAL AND METHODS

The series consisted of the *ganglion cervicale craniale*, the *ganglion thoracale primum*, and the *ganglion mesentericum craniale* of cattle, horses, dogs, and cats. The material was removed as soon as the animals were killed. It was fixed in Bouin's solution or 10 per cent formalin. After paraffin embedding applicable pieces were cut at 5—10 micra sections. A modification of the aldehyde-fuchsin method (12) was employed after deparaffinization and hydration through xylene and alcohol series.

RESULTS

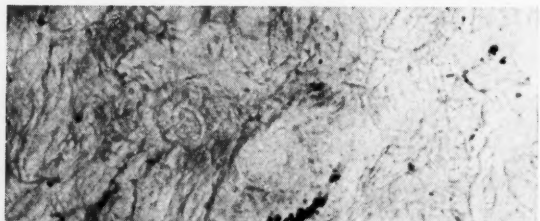


Fig. 1. — Section from the *ganglion cervicale craniale* of a cow. Aldehyde-fuchsin method. × 400.

Fig. 2. — Group of cells from the *ganglion thoracale primum* of a dog. Aldehyde-fuchsin method. × 220.

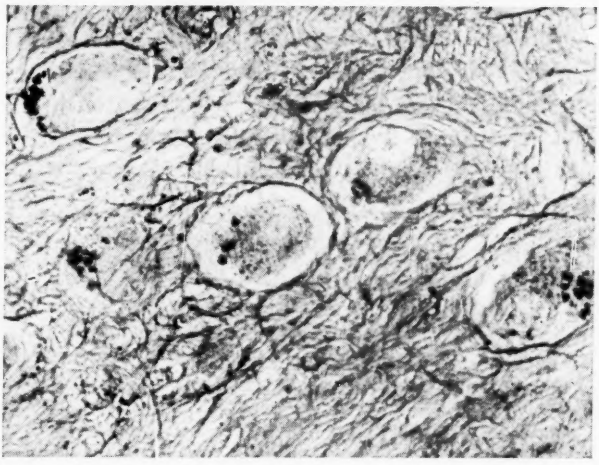


Fig. 3. — *Ganglion mesentericum craniale* of a horse. Aldehyde-fuchsin method. × 400.

In the ganglion cells the aldehyde-fuchsin positive material was to be found in the shape of small granules (Fig. 1—3). The similar observations could be made on every ganglion of all the animals of this investigation. The ganglion cells were not found to be alike. It could be observed that the amount of the material in different cells varied considerably. In some cells granular material was entirely absent, in the others only a small amount was found. The

granules were in those cells which contained the largest amount of material in semilunar aggregations at one of the poles of the cell in the peripheric zone of the cytoplasm. In general, the granulation occurred only in some areas of the cytoplasm. It seemed to be mostly in the periphery. Some granules could be found between the ganglion cells outside the cell bodies in the immediate neighbourhood of the cells.

DISCUSSION

The aldehyde-fuchsin positive material is apparently not homogenous at all. This opinion is supported by the fact that this material was to be found in various tissues (8). In the hypothalamic-hypophysial system of the central nervous system it is identical with Gomori's substance, which contains the principles of the posterior lobe of the hypophysis (1). The aldehyde-fuchsin positive material occurred in the shape of granules in some parts of the central nervous system *e.g.* in the nucleus of the third cranial nerve (17) and in the Purkinje cells (18). Whether the aldehyde-fuchsin material in these cells is the result of the secretion of the nerve cells or not, is difficult to decide without comprehensive investigations *e.g.* as to the eventual physiologic effect of the secret. The answer to this problem awaits a more detailed study. Nothing positive as to the aldehyde-fuchsin positive material of the autonomic ganglion cells can be said, either.

SUMMARY

The series consisted of certain ganglia of the autonomic nervous system of cattle, horses, dogs, and cats. It was investigated according to Gomori's aldehyde-fuchsin method. The aldehyde-fuchsin positive material was to be found in the shape of granules in every ganglion of all the species of this investigation. The nature of the material was discussed.

REFERENCES

1. Bargmann, W.: Colston Papers 1957:*8:*11.
2. Biscop, G.: Z. Zellforsch. 1948:*34:*141.
3. Dawson, A. B.: Anat. Rec. 1953:*115:*63.
4. Eichner, D.: Z. Zellforsch. 1951:*36:*293.
5. Eichner, D.: Z. Zellforsch. 1952:*37:*274.
6. Ehlers, P.: Anat. Anz. 1951:*98:*24.
7. Gaupp, R.: Z. Neur. 1937:*160:*357.
8. Halmi, N., and Davies, J.: J. Histochem. Cytochem. 1953:*1:*447.
9. Herzog, E.: Beitr. path. Anat. 1938:*101:*390.
10. Kito, T.: Folia anat. jap. 1954:*26:*221.
11. Kivalo, E., and Talanti, S.: Acta endocrin. 1957:*26:*128.
12. Landing, B., Hall, H., and West, C.: Lab. Investigation 1956:*5:*256.
13. Lehmann, H. J., and Stange, H. H.: Z. Zellforsch. 1953:*38:*290.
14. Meyer, E. R.: Beitr. path. Anat. 1950:*111:*373.
15. Müller, W., and Walter, W.: Z. Anat. 1955:*118:*348.
16. Scharrer, E., and Scharrer, B.: v. Möllendorffs Handbuch d. mikrosk. Anat. d. Menschen. Bd. VI, 5, 953, Heidelberg 1954.
17. Sloper, J. C.: J. Anat. (London) 1955:*89:*301.
18. Talanti, S., and Ki valo, E.: Ann. Med. exp. Biol. Fenn. 1958:*36:*375.
19. Unger, K.: Anat. Anz. 1951:*98:*13.

FROM THE DEPARTMENT OF ANATOMY, VETERINARY COLLEGE, HELSINKI, AND FROM THE DEPARTMENT OF ANATOMY, UNIVERSITY OF TURKU

OBSERVATIONS ON THE DISTRIBUTION OF ACID PHOSPHATASE IN THE BRAIN STEM OF THE RAT

by

S. TALANTI, L. LEHTINEN, and E. KIVALO[1]

(Received for publication July 12, 1958)

In a previous investigation, two of the present authors have performed a histochemical comparison with regard to the acid phosphatase activity in the cerebellum of the rat, particularly between its various cell groups, and they have compared this activity with the acid phosphatase of the choroid plexus in the fourth ventricle (2). In 1952, Leduc and Wislocki investigated the histochemical localisation of certain enzymes in the structures comprising the haematoencephalic barrier of the rat (3). In the results presented by them, these authors state that they have observed a strong acid phosphatase activity particularly in the cytoplasm of the motor cells of the brain stem and a distinct activity in the cytoplasm and the nuclei of the cells in the area postrema. In the present investigation the objective was to institute a comparison between the different parts of the brain stem of the rat with regard to their acid phosphatase activity, i.e., primarily with regard to its histochemically demonstrable general distribution.

MATERIAL AND METHODS

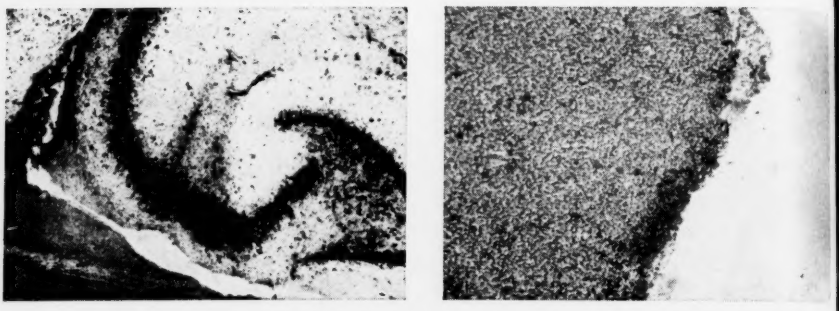
Altogether eight adult white rats, half male and half female, were used for this investigation. They were rapidly killed by decapi-

[1] Aided by a grant from the State Commission for Sciences.

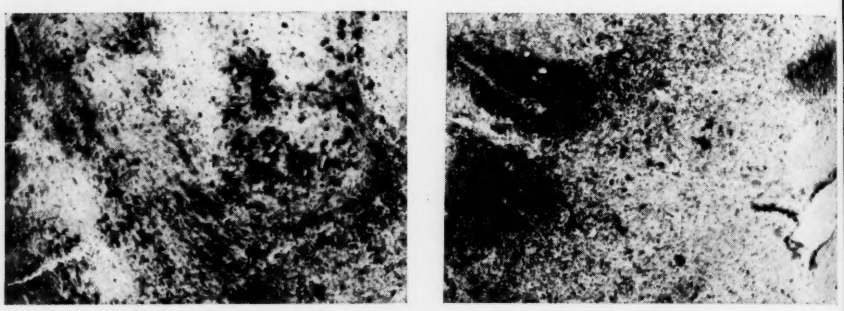
tation. Subsequently, the brain and the medulla oblongata were prepared out rapidly and treated for the histochemical demonstration of phosphatase according to Eränkö's modification as was done in the preceding work (1, 2). From part of the material cuts were made with the freezing microtome in the usual manner by cutting the tissues transversally, whereas part of the material was cut in the horizontal and sagittal planes.

RESULTS AND DISCUSSION

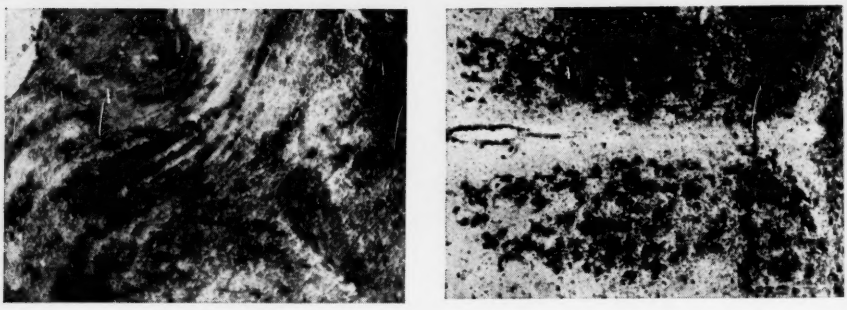
On investigation the acid phosphatase activity in the various parts of the brain stem with the rat, primarily a comparison was performed to that strong activity which was observed to exist in the choroid plexus, as has been shown in earlier investigations (3). In fig. 1 a strong activity can be seen in the plexus chorioideus of the lateral ventricle, and the same photograph also shows the amygdaloid nucleus and part of the cortex of the temporal lobe. — On examining the various parts of the mesencephalon, it can be observed that throughout the whole cut surface of the tissue the structure of the different parts is clearly visible. This can also be noted in the other parts of the brain which were investigated. It shows that in the longitudinal and transversal cuts of the axons the acid phosphatase activity is made visible although the general impression is one of fairly feeble strength. Slightly stronger activity is observed around the aqueductus Sylvii in the region of the substantia grisea. On the other hand the crura cerebri and the colliculi are faintly active (fig. 2). The substantia nigra is not much different from its surroundings but the red nucleus (fig. 3) and the surround ingformatio reticularis are more strongly active, particularly so the cells of the red nucleus. However, the cells of the oculomotor nucleus and the area in their immediate vicinity are most active of all in this region of the nervous system (fig. 4). — In the metencephalic region, activity of corresponding strength is observed particularly in the area of the nuclei pontis (fig. 5). Elsewhere, there only the general structure of the tissue is visible except in places where nuclei of the various cerebral nerves are encountered and where at their sites a strong acid phosphatase activity is obvious. — In the area of myelencephalon there is still a strong activity in the nuclei of the cerebral nerves (fig. 6), but,



1



3



5

Fig. 1. — The acid phosphatase activity of the plexus choroideus of the lateral ventricle and in amygdaloid nucleus of the rat. × 30.

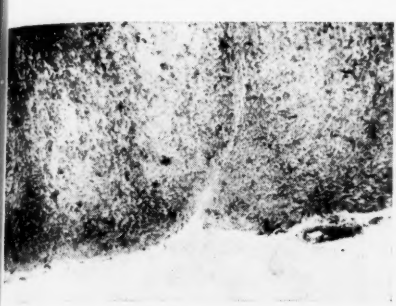
Fig. 2. — The poor acid phosphatase activity in the colliculus rostralis of the rat. × 120.

Fig. 3. — The acid phosphatase activity of the nucleus ruber and in the formatio reticularis of the rat. × 48.

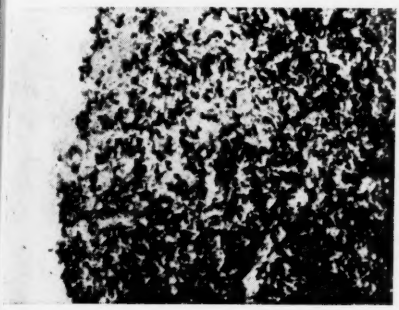
Fig. 4. — The acid phosphatase activity of the nucleus originis nervi oculomotorii of the rat. × 48.

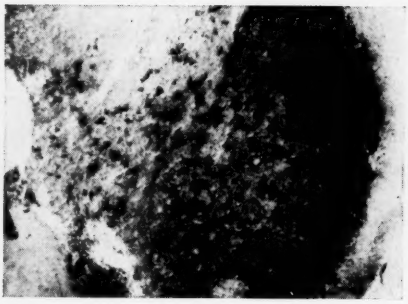
Fig. 5. — The acid phosphatase activity of the nuclei pontis of the rat. × 48.

Fig. 6. — The acid phosphatase activity of the nucleus nervi hypoglossi of the rat. Horizontal section. × 120.



8





10

Fig. 7. — The acid phosphatase activity of the decussatio pyramidum of the rat. × 48.

Fig. 8. — The acid phosphatase activity of the corpus restiforme and in the nucleus olivaris. × 120.

Fig. 9. — The strong acid phosphatase activity of the area postrema of the rat. × 120.

Fig. 10. — The acid phosphatase activity of the formatio reticularis in the medulla oblongata of the rat. × 48.

as was already observed in the foregoing, the cross-section of the cortico-spinal tract, for instance, shows fairly poor activity also here at the decussation of the pyramids (fig. 7). In areas where nerve cells are encountered abundantly, such as in the neighbourhood of the corpus restiforme and at the olivary nucleus, the activity is stronger (fig. 8). Particular attention is called to the region of area postrema (fig. 9), where a stronger acid phosphatase activity exists than in its immediate surroundings, as has already been observed before (3).

In the cross-cut of the myelencephalon, as well as in its longitudinal sections, very strong activity is noted in the region of

the formatio reticularis, which is clearly different from that of the formatio reticularis of the mesencephalon higher up. Furthermore, one can particularly observe here (fig. 10) the strong acid phosphatase activity in the marginal region of the formatio reticularis, where the activity is of such strength even when produced with a short incubation time that it is not possible to distinguish cell structures but the precipitated sulphide of lead has been deposited as a thick, coherent zone which is encountered in all cross-cuts in the entire region of the medulla oblongata. — However, even this activity does not surpass that in the choroid plexuses, but it is at least of the same order as the activity demonstrable in the cells of the motor nuclei of the brain stem. The strong acid phosphatase activities of the formatio reticularis as well as the area postrema are indicative of their important significance, which continues even at this moment to be one of the most essential objects of study in the investigation relating to the central nervous system.

SUMMARY

A histochemical study has been made of the acid phosphatase activity in the brain stem of white rats and of its distribution in this region. It is observed that, as a consequence of the activities of varying strength, the entire structure of the brain stem is made visible by this method. The strongest activity was observable in the region of the motor nuclei of the brain stem, in that of the formatio reticularis of the medulla oblongata, and in that of the area postrema.

REFERENCES

1. Eränkö, O.: Ann. med. exper. et biol. Fenn. 1951:*29*:287.
2. Kivalo, E., and Lehtinen, L.: Ann. med. exper. et biol. Fenn. 1957: *35*:387.
3. Leduc, E. H., and Wislocki, G. B.: J. Comp. Neur. 1952:*97*:255.

FROM THE DEPARTMENT OF MEDICAL CHEMISTRY, UNIVERSITY OF TURKU

SYNTHESIS OF β-AMINOPROPIONITRILE-1-^{14}C

by

TAINA TUOMINEN, KALE JUVA, and EINO KULONEN

(Received for publication June 6, 1958)

The intention was to study the metabolism of labelled β-aminopropionitrile (the toxic substance in *Lathyrus*-seeds). A corresponding investigation has been done with aminoacetonitrile (1), but the results are not necessarily relevant to the impairment of connective tissue synthesis and large part of the products remained unidentified. The synthesis of β-aminopropionitrile was modified from the described procedures (2, 3). However, due to the instability of the materials this approach seems impracticable. The chromatographic behaviour of the β-aminopropionitrile was also noted.

EXPERIMENTAL

Synthesis. — Concentrated solution of ammonia (pure grade) was heated at water bath up to +30°C. Acrylonitrile (Fluka A.G., pure) was allowed to flow from a separating funnel to the bottom of the two-necked reaction flask which was also connected to a reflux condenser. The optimal ratio acrylonitrile:ammonia (v/v) was found to be 1:10. Heat evolved and the mixture began to boil which was allowed for 5 min. The reaction flask was rapidly connected with a vacuum destillation equipment and the water and ammonia destilled off in reduced pressure. The mixture had to be heated, but care was taken that the temperature did not exceed +30°C. Destillation was continued at higher temperature in a smaller flask. Two fractions were obtained and the first corresponded to β-aminopropionitrile (b.p. 70°C. at 10 mm. Hg). The yield in several trials was about 40%.

The synthesis of labelled β-aminopropionitrile was conducted similarly. The acrylonitrile-1-^{14}C (1 mc., 3.7 g.) was obtained from The Radiochemical Centre (Amersham, Bucks., England). This compound was already partially polymerized before use (28 days) and carrier (5 ml. acrylonitrile) had to be added and before the final destillation about 10 ml. of β-aminopropionitrile. About 8.3 gm. of β-aminopropionitrile was obtained containing 11.7 μc. only (determined by The Radiochemical Centre).

The β-aminopropionitrile has been completely stable at +4°C. for several months. However, the weakly active β-aminopropionitrile solidified completely at the room temperature in 5 weeks, but was still soluble in water. When the basic groups were titrated from the solution, only about one fourth from the expected was found.

These two products were studied with two-dimensional paper chromatography (Fig. 1), using butanol-acetic acid-water (4:1:5) and ammoniacal phenol-water as solvents. The β-aminopropionitrile could also be observed in the urines of sweet pea-intoxicated rats (4). The radioactive preparation yielded only a bluish purple spot of β-alanine. The green spot was almost absent.

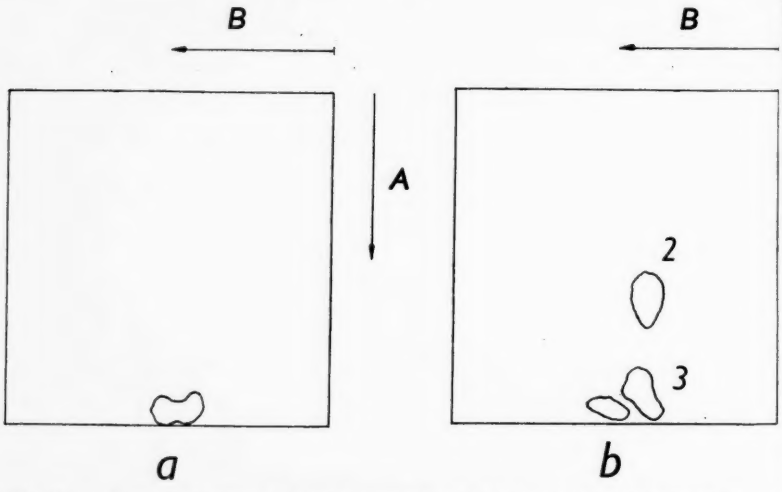


Fig. 1. — Chromatograms of β-aminopropionitrile (a) and similarly synthetized β-aminopropionitrile-1-^{14}C (b)
a) β-aminopropionitrile, brightly green; b) 2 = β-alanine; 3 = brown shadow (staining with 0.2% ninhydrin). A = phenol-water-ammonia, B = butanol-acetic acid-water

The non-labelled β-aminopropionitrile was potent as lathyrogenic agent in rats in a dose of 30 mg. per day. The solidified and dissolved material (in dose of 100 mg. per day) was completely inactive.

DISCUSSION

The instability of the acrylonitrile seems to be due to the radiation. The β-aminopropionitrile carrier was completely stable, but when some water had been added, it solidified similarly as the labelled product. Since the activity was extremely low, this reaction seems thus to be independent from the radiation.

These handicaps render this synthesis rather impracticable for the work with the labelled β-aminopropionitrile but for the preparation of the non-labelled compound it is simple and convenient.

SUMMARY

The synthesis of β-aminopropionitrile-1-^{14}C is described.

Acknowledgement. — This experiment forms a part of a program which has been supported by the State Board of Science and Sigrid Jusélius Stiftelse. Also the directors of the Radiochemical Centre have shown much courtesy and generosity to us.

REFERENCES

1. Ponseti, I. V., Wawzonek, S., Franklin, W. E., Winnick, R. E., and Winnick, T.: Proc. Soc. Exptl. Biol. Med. 1956:*93*:515.
2. Buc, S. R., Ford, J. H., and Wise, J. H.: J. Am. Chem. Soc. 1945:*67*:92.
3. Ford, J. H., Buc, S. R., and Grenier, J. W.: Ibid 1947:*69*:844.
4. Yli-Pohja, M., Tuominen, T., and Kulonen, E.: Clinica Chimica Acta 1958:*3*:397.

FROM THE DEPARTMENT OF PATHOLOGICAL ANATOMY, UNIVERSITY OF TURKU

THE MODIFYING EFFECT OF HYDROCORTISONE ACETATE ON THE CHLORPROMAZINE-CAUSED CHANGES OF THE ADRENAL CORTEX IN THE WHITE RAT

by

ARMO HORMIA[1], SEPPO KANGAS, and MARTTI HORMIA

(Received for publication June 17, 1958)

In an earlier paper (4) the effect of large chlorpromazine doses on the adrenal cortex of the male white rat was investigated. It was shown that prolonged chlorpromazine medication with progressively increasing doses caused an increase of the weight of the adrenals. Furtherly, in the histological picture of the adrenal cortex typical changes were seen: hypertrophy of the entire cortex, decrease of the lipid content of the cells, disappearance of the transitional zone and an abundance of sinusoids in the zona reticularis. These changes manifested themselves parallel with the deterioration of the general condition of the animals. Thus they were only slightly seen in the group treated with slowly increasing doses of chlorpromazine (increased by 5 mg/kg body weight daily) but were clearly seen in the group treated with rapidly increasing doses (increased by 25 mg/kg body weight daily). The simultaneous application of small doses of corticotrophin (2 IU/kg body weight daily) had no essential effect on the results. These changes were not to be seen after a single wery large dose of chlorpromazine (600 mg/kg body weight). Thus the prolonged way of medication was observed to be an essential presupposition to the appearance of the changes. The results were interpreted to prove that chlorpromazine in prolonged use with large doses had acted as a nonspecific stressor to the organism.

[1] Sponsored by a grant of the Sigrid Jusélius Stiftelse.

Corticoids, especially glucocorticoids are effective in preventing an adrenal enlargement caused by stress (1, 2, 6, 7). Therefore, in order to throw further light on the mode of action of chlorpromazine, the following experiments were performed. They investigate the effect of hydrocortisone acetate on these chlorpromazine-caused changes of the adrenal cortex refered above.

MATERIAL AND METHODS

The effect of simultaneous hydrocortisone acetate medication on the chlorpromazine-caused changes of the adrenal cortex was studied by male white rats. Also a parallel series with cortrophine was performed. 10 rats served as controls. Three groups of 10 rats in each were treated with chlorpromazine using rapidly increasing doses. On the first day the dose was 20 mg/kg body weight and the dose was increased by this same amount daily. 10 rats (group I) were treated with chlorpromazine alone, to 10 rats (group II) an accessory daily dose of 15 IU/kg body weight corticotrophin ('Reacthin' Leo) was given, and to 10 rats (group III) 25 mg/kg body weight hydrocortisone acetate (Lääke Oy). The drugs were given subcutaneously. The general condition of the animals was followed up. For this purpose the rats were weighed before and during the period of medication. In the beginning of the experiment the animals were 90 days of age. The medication was continued during 14 days. After this the animals were killed. Immediately after killing the suprarenals of each animal were removed and weighed in a torsio balance. For the study of the lipoid content of the adrenal cortex, one of the suprarenals was fixed for 24 hours in neutralized 4% formol. 10 μ frozen sections were cut and stained with the Sudan III method. Morphological study was performed after a 5-hours fixing in Zenker's solution and after staining with haematoxylin and eosin.

RESULTS

Fig. 1 gives the changes in the body weight of the animals in the different groups during the period of medication. It shows that during the first half of the treatment no great differences between the experimental and control groups were seen. After this, the body

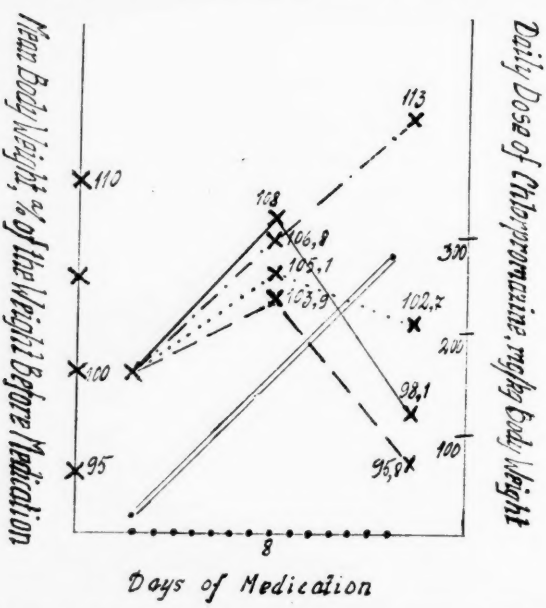


Fig. 1. — The effect of chlorpromazine alone and in combination with ACTH or hydrocortisone acetate on the mean body weight of the male rat. Mean body weight:

× —·— × = Control group.
× ——— × = Group I, treated with chlorpromazine alone.
× ····· × = Group II, treated with chlorpromazine and ACTH combined.
× - - - - - × = Group III, treated with chlorpromazine and hydrocortisone acetate combined.
·═══· = Daily dose of chlorpromazine.

weight began rapidly go down in the experimental groups. Compared with the control group the group I, treated with chlorpromazine alone, had deteriorated by 13%, the group II, treated with chlorpromazine and ACTH, by 9%, and the group III, treated with chlorpromazine and hydrocortisone acetate, by 15%. In accordance with this, the outlook of the animals showed no clear signs of depressed vitality during the first half of the treatment, even if the spontaneous motor activity was diminished as a sign of sedation. During the second half of the treatment the general condition of the animals began to weaken and now an interesting difference between the experimental groups showed itself. In clear contrast to the other groups the sedative effect of chlorpromazine in the group receiving hydrocortisone acetate still remained moderate,

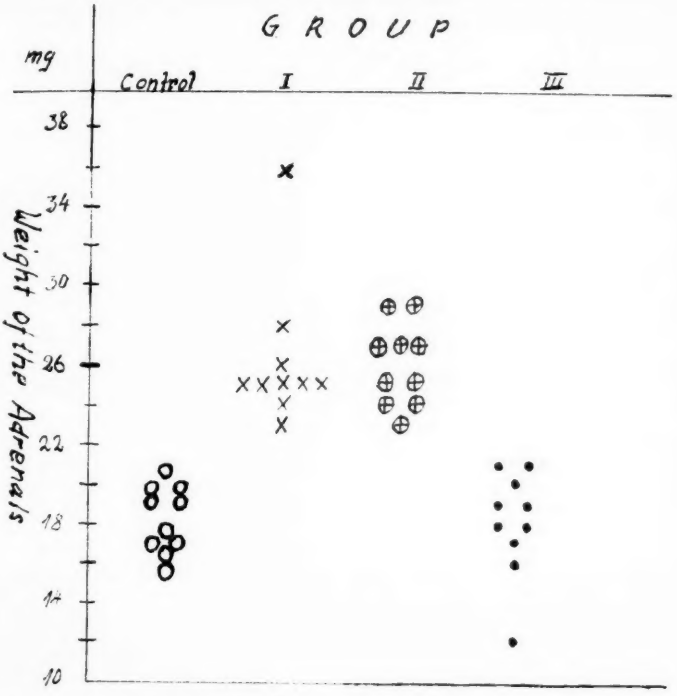


Fig. 2. — The effect of chlorpromazine alone (group I), and in combination with ACTH (group II) or hydrocortisone acetate (group III) on the weight of the adrenals of the male rat. The marks indicate the mean weight of the suprarenal glands of the individual animals in different groups.

the animals even behaved themselves aggressively. The difference between the groups could also be demonstrated by suspending animals by their front paws from a horizontal wire. At the end of the medication the group I, receiving chlorpromazine was as a rule not more able to climb on the wire. The group II, receiving chlorpromazine and ACTH combined proved in this traction test to be still more inert than the group receiving chlorpromazine alone.The group III, receiving chlorpromazine and hydrocortisone acetate combined, usually immediately climbed on the wire. Also the outlook of the animals showed corresponding differences. Apart from the fact that the loss of weight during medication was biggest in the group receiving hydrocortisone acetate, no spontaneous bleedings from mucous membranes or convulsions were to bee seen in this group, in the two other groups they were present to an equal extent.

Fig. 2 gives the weights of the suprarenals in different groups. It shows that the suprarenals had considerably gained in weight in the group I, receiving chlorpromazine alone, and in the group II, receiving chlorpromazine and ACTH combined. The increase is seen to be almost identical in these two groups. The group III, receiving chlorpromazine and hydrocortisone acetate, on the other hand, does not differ from the control group. The mean weight of the suprarenals in the different groups was: Control group = 18.1; mg; group I = 26.3 mg; group II = 25.6 mg; group III = 18.1 mg.

By studying microscopically the sections stained with Sudan III, in the control group the glomerulosa contained numerous small Sudan-positive droplets. The transitional zone was well formed in all cases. The droplets in the cells of zona fasciculata were rather large, and in the outer parts of the fasciculata sudanophilia was stronger than in the inner parts. Zona reticularis was rather weakly sudanophil (Fig. 3). In the group treated with chlorpromazine alone sudanophilia was rather evenly distributed through the whole cortex. No transitional zone was seen and zona glomerulosa seemed to be very thin (fig. 4). In the group treated with chlorpromazine and ACTH the distribution and amount of sudanophilia did not essentially differ from the preceding group (fig. 5). In the group treated with chlorpromazine and hydrocortisone acetate the distribution and amount of sudanophilia was in most cases like that in the control group (fig. 6). In some cases, however, zona glomerulosa was very thin, no transitional zone was to be seen and sudanophilia was rather evenly distributed through the whole cortex.

In haematoxylin-eosin sections zona glomerulosa was rather thick in the control group. The transitional zone was clearly seen in all cases and its cells were rather eosinophil and the nuclei were situated close together. In zona fasciculata the cells were clear and vacuolated and the sinusoids in zona reticularis were small. Eosinophilia was strongest in the outer parts of the fasciculata, diminishing gradually towards the inner parts (fig. 7). In the group treated with chlorpromazine alone zona glomerulosa was very thin, hardly to be seen at all. No transitional zone was to be seen. The whole cortex was hypertrophied, hypertrophy having taken place mainly in zona fasciculata and reticularis. The cells in zona fasciculata were rather dense and only some small vacuoles were seen in

Fig. 3.

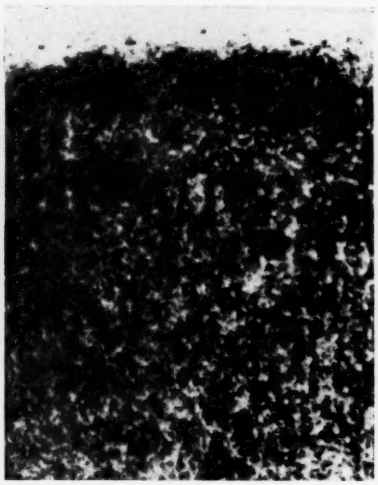


Fig. 4.

Fig. 3. — Photomicrograph showing sudanophilia in the adrenal cortex of the control group. The sudanophobic zone is clearly seen. 350 ×, Sudan III.
Fig. 4. — Photomicrograph showing sudanophilia in the adrenal cortex of the group treated with rapidly increasing doses of chlorpromazine. No sudanophobic zone is seen and zona glomerulosa can not be clearly distinguished. Sudanophilia is distributed through the whole cortex more evenly than in the control group. 350 ×, Sudan III.

Fig. 5.

Fig. 6.

Fig. 5. — Photomicrograph showing sudanophilia in the adrenal cortex of the group treated with chlorpromazine and ACTH. No essential difference to the preceding photomicrograph is seen. 350 ×, Sudan III.
Fig. 6. — Photomicrograph showing sudanophilia in the adrenal cortex of the group treated with chlorpromazine and hydrocortisone acetate. No essential difference to the fig. 3 is seen. 350 ×, Sudan III.

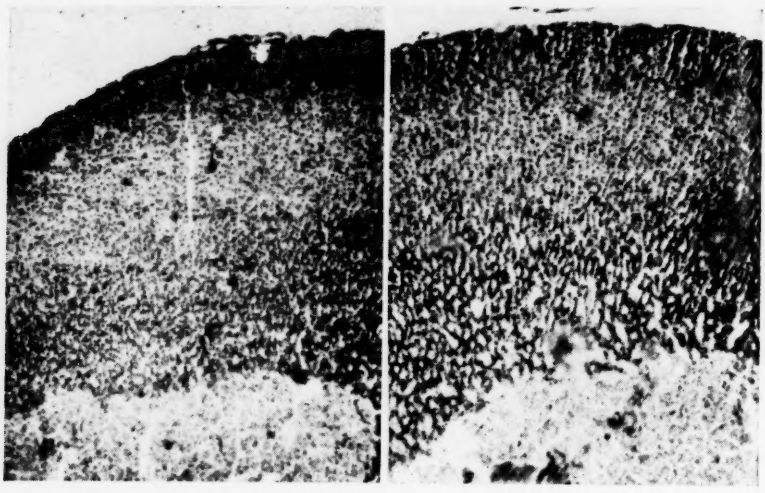
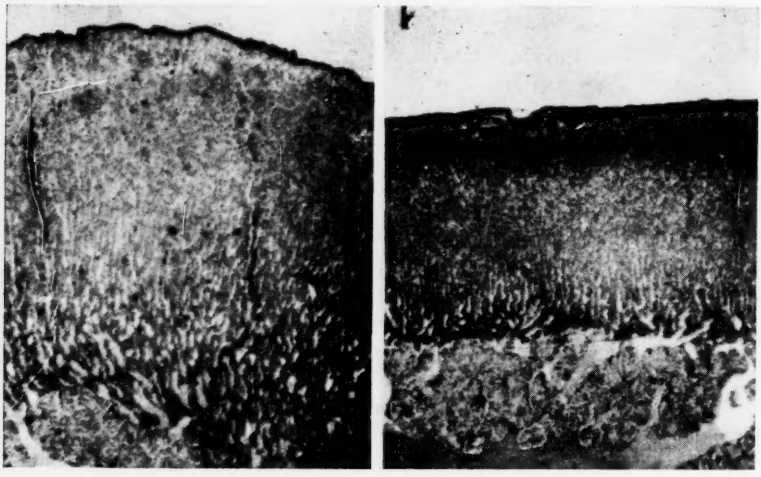


Fig. 7. Fig. 8.

Fig. 7. — Photomicrograph showing adrenal cortex in the control group. Zona glomerulosa is rather thick and the transitional zone is seen as a dark line between zona glomerulosa and fasciculata. 160 ×, haematoxylin — eosin.
Fig. 8. — Photomicrograph showing adrenal cortex in the group treated with rapidly increasing doses of chlorpromazine. Zona glomerulosa is hardly to be distinguished, no transitional zone is seen and zona glomerulosa and fasciculata are hypertrophied. 160 ×, haematoxylin — eosin.



Fig, 9. Fig. 10.

Fig. 9. — Photomicrograph showing adrenal cortex in the group treated with chlorpromazine and ACTH. No essential difference to the preceding photomicrograph is seen. 160 ×, haematoxylin — eosin.
Fig. 10. — Photomicrograph showing adrenal cortex in the group treated with chlorpromazine and hydrocortisone acetate. No essential difference to the control group is seen. 160 ×, haematoxylin — eosin.

them. The sinusoids in zona reticularis were rather large and blood-filled (fig. 8). In the group treated with chlorpromazine and ACTH the histological picture did not essentially differ from the preceding group (fig. 9). In the group treated with chlorpromazine and hydrocortisone acetate the transitional zone was clearly seen in most cases. Also in other respects the histological picture was much like that in the control group (fig. 10). In some cases, however, the whole cortex seemed to be hypertrophied, zona glomerulosa was very small and no transitional zone was to be seen or it was indistinct.

DISCUSSION

As is known, ACTH causes hypertrophy of the adrenal cortex (3, 5, 9). By giving ACTH to animals under stress, the hypertrophy of the adrenal cortex is larger than that caused by stress alone (cf. also 8). The fact that the accessory application of ACTH in the experiments presented above neither intensified the increase of the weight of the adrenals nor the histological signs of stress caused by chlorpromazine, shows that the chlorpromazine-caused stress has been maximal. Hydrocortisone acetate inhibited the hypertrophy of the adrenal cortex caused by chlorpromazine. This is in accordance with the earlier observations concerning the ability of corticoids to inhibit the signs of stress in the weight of suprarenals. In the histological picture of the adrenal cortex, however, signs typical to stress could be seen in some cases. Presumably this can be interpreted as an expression of the fact that the dose of hydrocortisone acetate has been a little too small to inhibit the signs of stress in all cases.

The observation that also the sedative effect of chlorpromazine in the group receiving hydrocortisone acetate was of less degree than in the group treated with chlorpromazine alone points to the direction as if the sedative effect of chlorpromazine partly were due to its stress-producing effect. Further investigations are needed to clear up this presumption.

SUMMARY

The modifying effect of simultaneously applicated hydrocortisone acetate on the signs of stress in the adrenal cortex caused

by a massive chlorpromazine medication was investigated by male white rats. For further evidence a parallel series with ACTH was performed. 10 rats served as controls.

Each experimental group contained 10 animals. One group was treated with chlorpromazine alone. The drug was given in a progressively increased dosage, increased by 20 mg/kg body weight daily. To the other group an accessory daily dose of 15 IU/kg body weight ACTH was given, and to the third group 25 mg/kg body weight of hydrocortisone acetate. The drugs were given subcutaneously and the medication was continued during 14 days.

The weight of the adrenals had increased in the group receiving ACTH in the same degree as in the group receiving chlorpromazine alone. Also the signs of stress in the histological picture of the adrenal cortex were equal. This was considered to be an expression of the fact that the stress caused by chlorpromazine had been maximal. The weight of the suprarenals in the group receiving hydrocortisone acetate did not differ from that in the control group and in the histological picture of the adrenal cortex signs of stress were present only in some cases. This result was considered to be in accordance with the earlier observations concerning the ability of corticoids to counteract the manifestation of the signs of stress in the adrenals. The sedative effect of chlorpromazine was mildest in the group receiving hydrocortisone acetate. This was presumed to point to the direction that the sedative effect of chlorpromazine partly were due to its stress-producing character.

REFERENCES

1. Dalton, A. J., Dosne, C., and Selye, H.: Anat. Rec. 1940:*76:*85.
2. Dosne, C., and Selye, H.: Federation Proc. 1942:*1:*21.
3. Eränkö, O.: Acta endocr. 1955:*18:*189.
4. Hormia, M., and Hormia, A.: Ann. Med. Exper. Fenn. 1958:*36:*Suppl. 3.
5. Landing, B. H., and Feriozi, O.: J. Clin. Endocr. 1954:*14:*1023.
6. Selye, H.: Canad. M. A. J. 1940:*42:*113.
7. Selye, H.: The Physiology and Pathology of Exposure to Stress. Montreal 1950.
8. Selye, H., and Collip, J. B.: Endocrinology 1936:*20:*667.
9. Vasama, R., Kalliomäki, L., Vasama, R., and Näätänen, E.: Ann. Med. Exper. Fenn. 1957:*35:*Suppl. 1.

FROM DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI, AND THE RHEUMATISM FOUNDATION HOSPITAL, HEINOLA

INCIDENCE OF THE AGGLUNATION ACTIVATING FACTOR IN RHEUMATOID ARTHRITIS

SOME OBSERVATIONS

by

H. JULKUNEN, V. LAINE, H.-M. KOSKINEN, and A. TIILIKAINEN

(Received for publication June 24, 1958)

As shown by R. Grubb, and Waller and Vaughan (1956), the serum of rheumatoid arthritis patients is in some instances able to agglutinate Rhesus positive red cells coated with selected incomplete anti Rhesus serum.

According to Grubb, the serum of about a third (37%) of rheumatoid arthritis patients has this property. These cases are the ordinary typical and often rather pronounced cases of both sexes, showing both high and low erythrocyte sedimentation rates. The property has been found before, during, and after steroid or gold treatment. Grupp also states that it can remain for several years, but does not know how early in the course of the disease it appears.

This property has been found in one healthy person published so far.

Grubb also found that the Waaler-Rose test was always positive in cases where this property was to be found.

Since the reaction of AAF (agglunation activating factor) has been studied in a series of rheumatoid arthritis patients at the Rheumatism Foundation Hospital, we consider it worth while to publish our clinical findings.

The series consisted of 137 cases of clinically verified cases of rheumatoid arthritis (ARA criteria 1957), 24 male and 113 female.

Sensitized 0 Rh positive red cell agglutination (SORA) was tested in each case (Grubb, 3[1]). The Waaler-Rose test (Rose *et al.*, 1948) was made on the same blood samples using the modification developed by Wager (Wager 1950). In 124 instances serum electrophoresis was performed.

RESULTS

As seen in Table 1 the SORA was positive in 18.2% of the cases. No correlations were to be found between the age groups, the duration of the disease and the sex distribution. However, the stage distribution of SORA positive cases shows a tendency to be more often positive in advanced cases. About the duration of the disease we learn that this agglutinating property can be found as early as less than one year after the onset of the disease. Table 2 shows the distribution of SORA according to the duration of the disease.

Further, it is to be seen in Table 1 that there is no direct correlation between SORA positivity and the grade of ESR. No correla-

TABLE 1

COMPARISON BETWEEN SENSITIZED 0 RHESUS POSITIVE RED CELL AGGLUTINATION TEST (SORA+) ABD NON-AGGLUTINATING (SORA—) PATIENTS

	Number of Cases	Mean Age	♀	♂	Stage I	Stage II	Stage III	Stage IV	Duration of Disease Years	Mean E.S.R. mm.	Waaler-Rose Titer ≥16	Waaler-Rose Titer ≥32
SORA +	25 18.2%	42.3 7—54	22	3	8%	24%	32%	36%	8.4 (1—28)	32.4	56%	32%
SORA —	112	33.7 7—58	91	21	16.2%	16.9%	54.4%	12.5%	6.1 (¼–38)	43.1	59%	36%

TABLE 2

SYSTEMIC INVOLVEMENTS IN SORA+ AND SORA— GROUPS

	Number of Cases	Leukopenia	Eosinophilia	Le-Cells +	Nephropathia	Hepato- or Splenomegaly	Dermatitis	Carditis	Oedema	Pathol. Liver Function Tests	Severe Disease in Biopsy	Wasserman Reaction +	Total Signs
SORA +	(25)	2	1	3	1	1	2	—	—	3	3	1	17=68%
SORA —	(112)	6	3	3	3	3	2	2	6	11	3	2	44=40%

[1] As controls are used samples of rheumatoid serum kindly provided by Dr. R. Grubb. Lund, Sweden.

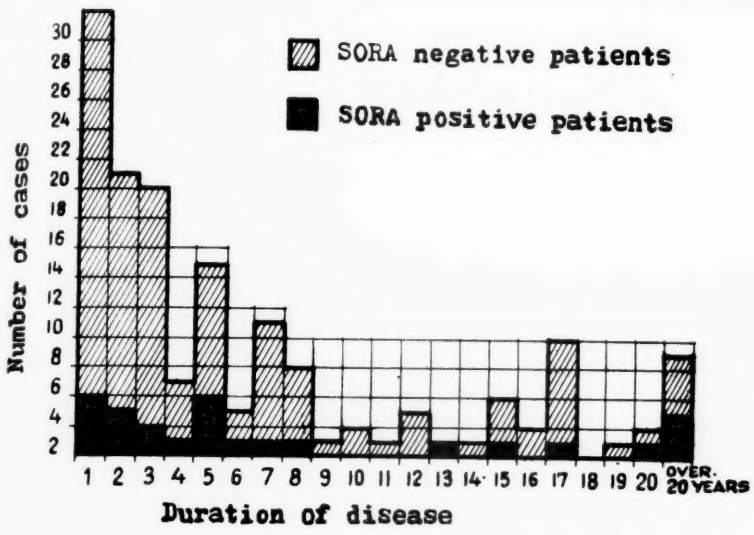


Fig. 1. — Distribution of total material and sensitized O Rh positive red cell agglutination (SORA+) patients according to duration of disease.

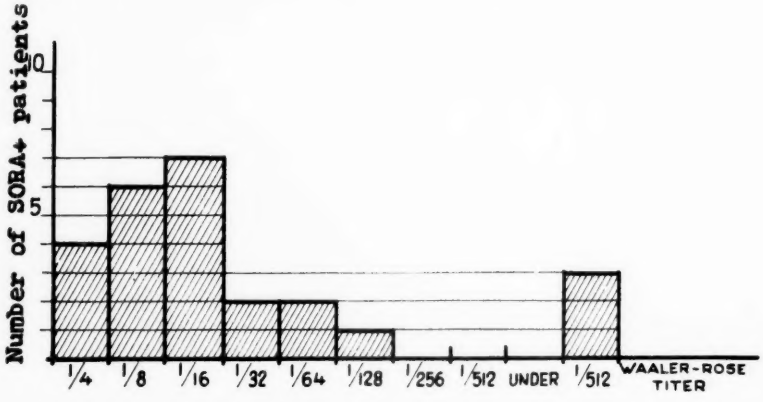


Fig. 2. — Correlation between SORA+ and Waaler-Rose titer.

TABLE 3

ELECTROPHORETIC PATTERNS OF SORA+ AND SORA— PATIENTS

	Number of Cases	Total Protein Mean	Alb./Glob.	Alb. %	α_1 %	α_2 %	β %	γ %
SORA +	21	7.41 %	1.05	51.5.	3.55	8.87	13.2	23.3
SORA —	103	7.60 %	0.95	48.2	3.23	9.58	13.3	25.7

tion has been found between SORA positivity and the positivity of the Waaler-Rose test, as Fig. 2 shows. In 10 out of 25 SORA positive cases the Waaler-Rose titer is below 1/16. This result differs clearly from that of Grubb's. However, the difference of the percentage of Waaler-Rose positives in both materials is very great, giving rise to the supposition of underlying differences in techniques.

In Table 2 we have collected some criteria which can be held as signs of systemic involvements in connection with rheumatoid arthritis, and which characterize the severity of the disease. It seems to be evident that there are more of these severe cases with systemic signs in the SORA-positive cases than in the SORA-negative cases.

In Table 3 the results of electrophoretic patterns of both SORA-positives and negatives are shown. As seen, no statements can be made of these. The SORA-positive cases do not show greater deviations from the normal electrophoretic pattern than the SORA-negative cases.

Summarizing the results it was possible to find, in a series of 137 cases of verified rheumatoid arthritis, sensitized 0 Rh positive red cell agglutination in 18%.

This agglutinating property can be found in early stages (1 year and less), as well as in advanced cases of rheumatoid arthritis.

In this series no consequent correlation has been found between the positive Waaler-Rose test: in 10 of the 25 SORA positive cases the Waaler-Rose test was negative.

Among the cases with the SORA property there seem to be more severe cases with systemic involvements than appear among the SORA-negative cases.

The paper electrophoretic pattern of serum proteins does not show any correlation to the positivity of the agglutinating property.

REFERENCES

1. Waller, V., and Vaughan, J.: Proc. Soc. exp. Biol. 1956:*92*:198.
2. Grubb, R.: Acta path. microbiol. scand. 1956:*39*:195.
3. Grubb, R.: Acta. path. microbiol. scand. 1956:*39*:390.
4. Grubb, R.: Acta rheum.scand. 1957:*3*:55.
5. Rose, H. M., Ragan, C., Pearce, E., and Lipman, M. O.: Proc. Soc. exp. Biol. 1948:*68*:1.
6. Wager, O.: The thesis, University of Helsinki 1950.

FROM THE WIHURI RESEARCH INSTITUTE, HELSINKI

URINARY EXCRETION OF 5-HYDROXYINDOLEACETIC ACID IN EXPERIMENTAL PULMONARY EMBOLISM IN RATS

by

H. FRICK

(Received for publication June 26, 1958)

The significance of neurohumoral factors in the development of the symptoms of pulmonary embolism has been the object of several investigations. In 1935 Schwiegk (14) showed that alterations in the pulmonary arterial pressure cause, through vagal stimulation, bradycardia and a fall of systemic arterial pressure. Some investigations have shown that symptoms caused by pulmonary embolism can be prevented or mitigated by vagotomy or by parasympathicolytic or ganglion-blocking substances (1, 3, 13). Other investigators, on the other hand, have not observed this effect (15, 19). The differing results may be, at least partly, due to the different materials and particle sizes used in the production of experimental embolism, to the different animal species used, and to the effects of anaesthesia, if used (8). Of humoral factors histamine has been mentioned, especially by French investigators (11, 17), as an important substance in the development of the symptoms of pulmonary embolism. Hochmeister (9), however, could not find any rise in the histamine content of the blood after pulmonary embolism.

Comroe *et al.* (4) were probably the first to pay attention to the possible role of 5-hydroxytryptamine (serotonin) in the genesis of the symptoms of pulmonary embolism. Smith and Smith (15) have compared the symptoms caused by experimental embolism

produced by a blood clot with those caused by serotonin injected into the pulmonary circulation and observed their similarity. According to Comroe *et al.* (4), Gaddum *et al.* (6), Borst *et al.* (2), Kottegoda and Mott (10), and Mott and Paintal (12), serotonin injected into the pulmonary circulation causes pulmonary hypertension, systemic hypotension, bradycardia, and bronchoconstriction. All of these phenomena are observed in experimental and clinical pulmonary embolism. Thus it may be supposed that serotonin, which occurs in thrombocytes and is released in the coagulation process (20), participates in the production of the symptoms of pulmonary embolism. The lung tissue also contains considerable amounts of serotonin (18).

Erspamer (5) has shown that the end product of serotonin metabolism is 5-hydroxy-3-indoleacetic acid (5-HIAA) which is excreted in the urine. De Gennes *et al.* (7) have observed that administration of reserpine, which is known to cause a decrease in the serotonin content of the thrombocytes, increases the urinary excretion of 5-HIAA.

In this work the effect of experimental pulmonary embolism in rats on the urinary excretion of 5-hydroxyindoleacetic acid was studied.

MATERIAL AND METHODS

Six male and two female rats of the Wistar strain weighing 240—470 g were used. The collection of urine took place in special cages equipped with funnels. The contamination of food and faeces was prevented by a wire netting in the funnels. Determinations of 5-HIAA were made from twenty-four hours' urine. The collection of urine was performed during two days before and one day after the production of pulmonary embolism. The spectrophotometric method of Udenfriend, Titus, and Weissbach (16) was used. When the amount of twenty-four hours' urine was more than 12 cc, determinations were made in duplicate. The blood clots used in the production of pulmonary embolisms were prepared by letting c. ½ cc of rat tail-tip blood clot. The blood clot was then suspended in a small amount of physiologic saline solution. The suspension was injected into the femoral vein. The procedure was performed under aether anaesthesia.

RESULTS

The daily amount of urine varied between 7.2 and 34.5 cc, which is of the same magnitude as that observed in the investigations of Erspamer (5) and de Gennes *et al.* (7). The mean daily amount of urine was 19.9 cc before the embolism and 12.9 after the embolism. The daily excretion of 5-HIAA in the urine is expressed in μg/100 g of body weight. Before the pulmonary embolism the daily excretion of 5-HIAA was 2.8—4.3 μg with a mean value of 3.5 μg; after the embolism it was 2.8—4.5 μg with a mean value of 3.4 μg. The methodical error was $\pm$ 0.4 μg. De Gennes *et al.* have used the same method in the determination of the excretion of 5-HIAA in rat urine and obtained a value of 1 $\pm$ 0.2 μg/100 g of body weight during 24 hours. The values obtained by Erspamer, 1.25—1.5 μg/ml, were determined by paper chromatography.

The values of individual rats are shown in the following Fig.

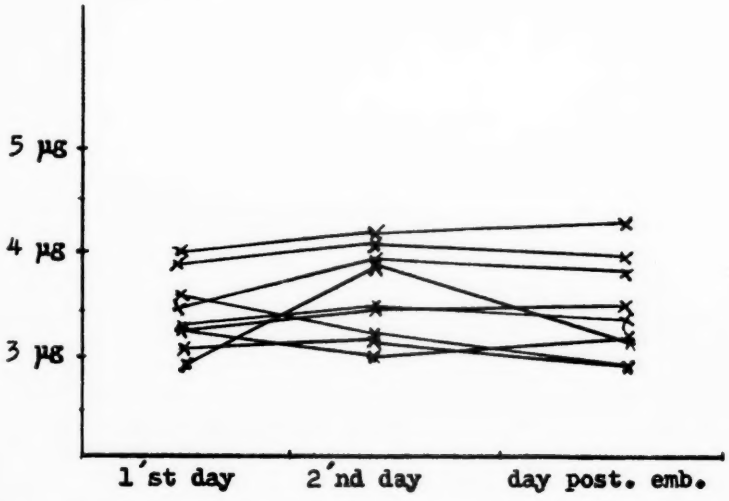


Fig. 1. — The daily excretion of 5-HIAA before and after pulmonary embolism.

The determination of the urinary excretion of 5-HIAA is one of the procedures which may be used in following the decomposition of serotonin. Since the excretion of urine of the experimental animals was small, the determinations were performed from the urine excreted during 24 hours. Rapid temporary alterations may

not, however, appear when the daily excretion is determined. Despite of great variations in the daily amount of urine, the excretion of 5-HIAA was, however, rather constant.

SUMMARY

The writer has determined the daily excretion of 5-hydroxy-3-indoleacetic acid in the urine of 8 rats of the Wistar strain during two days before and one day after the production of pulmonary embolism. No alteration could be found.

REFERENCES

1. Bein, H. J.: Experientia 1952:*8:*67.
2. Borst, H. G., Berglund, E., and McGregor, M.: J. Clin. Invest. 1957:*36:*669.
3. Byrne, J. J.: A.M.A. Arch. Surg. 1955:*70:*111.
4. Comroe, J. H. Jr., Van Lingen, B., Stroud, R. C., and Roncorini, A.: Am. J. Physiol. 1953:*173:*379.
5. Erspamer, V.: J. Physiol. 1955:*127:*118.
6. Gaddum, J. H., Hebb, C. O., Silver, A., and Swan, M.: Q. J. Exp. Physiol. 1953:*38:*255.
7. de Gennes, L., Tourneur, R., Aubert, P., Laudat, Ph., Moukhtar, M., and Baillet, J.: Presse Medic. 1957:*65:*1321.
8. Halmagyi, D. F. J.: Die Klinische Pathofysiologie des kleinen Kreislaufs. Fischer Verlag. Jena. 1957.
9. Hochmeister, W.: Arch. Exp. Path. Pharm. 1950:*210:*175.
10. Kottegoda, S. R., and Mott, J. C.: Brit. J. Pharm. 1955:*10:*66.
11. Moreau, P., Renais, J., and Scebat, L.: Arch. mal. coeur 1957:*50:*550.
12. Mott, J. C., and Paintal, A. S.: Brit. J. Pharm. 1953:*8:*238.
13. Pocidalo, J. J., and Damoiseau, B.: Presse Medic. 1953:*61:*1623.
14. Schwiegk, H.: Pflügers Arch. ges. Physiol. 1935:*236:*206.
15. Smith, G., and Smith, A. N.: Surg. Gyn. Obstetr. 1955:*101:*691.
16. Udenfriend, S., Titus, E., and Weissbach, H.: J. Biol. Chem. 1955: *216:*499.
17. Ungar, G., Grossiord, A., and Brincourt, J.: Compt. rend. Soc. Biol. 1935:*120:*632.
18. Weissbach, H., Waalkes, T. P., and Udenfriend, S.: Science 1957: *125:*235.
19. Williams, M. H. Jr.: Circulation Research 1956:*4:*325.
20. Zucker, M. B.: Proc. Int. Physiol. Congr. 1953:*76:*915.

FROM THE DEPARTMENT OF PATHOLOGY, SECTION II, UNIVERSITY OF HELSINKI, AND THE CENTRAL INSTITUTE OF RADIOTHERAPY, THE CENTRAL HOSPITAL, HELSINKI

EFFECTS OF INTRAPERITONEALLY INJECTED HOMOGENATES OF ROENTGEN IRRADIATED AND NON-IRRADIATED TUMOURS ON THE GROWTH OF HOMOLOGOUS AND HETEROLOGOUS TISSUES

by

A. VOUTILAINEN and V. K. HOPSU

(Received for publication July 5, 1958)

Mitosis-inhibiting agents have been assumed to occur in normal organs (3, 5, 6). Similar inhibitors are probably present also in tumour tissue (7). Injections of homogenates prepared from tumour tissue have been found to produce signs indicating growth inhibition (4).

The present study constitutes a further attempt to determine whether growth inhibition of the above kind can be indiced in the case of different tumours, especially heterologous malignant ones, and whether the growth-inhibiting agents are influenced by X-ray irradiation.

MATERIAL AND METHODS

Homogenates prepared from Walker carcinosarcoma and from Yoshida sarcoma were injected into rats bearing the former tumour. The homogenates used for the injections were prepared as follows:

This investigation was supported by President J. K. Paasikivi s Fund for Cancer Research, the Damon Runyon Memorial Fund (DRG—291) and the Sigrid Jusélius Foundation.

Walker's carcinosarcoma. — Twenty 3-month-old white rats were inoculated subcutaneously with this tumour. After 11 days irradiation of the tumours of 10 rats was started. The dose was 600 r daily for five successive days (0.5 Cu, 10 mA, 140 kV, H.V.L. 0.28 cm.Cu). The tumours regressed considerably during roentgen treatment: their average final weight was about 22 gm. as against about 70 gm. in the case of the non-irradiated controls. The tumours were excised 24 hours after the last treatment, dried in an dessicator at +3 C for two days, and powdered in a mortar; the powder was kept in the refrigerator. The amount of powder required was weighed immediately before injection and suspended in saline solution.

Yoshida sarcoma. — This tumour was inoculated into the femoral muscle of ten 3-month-old white rats. In 11 days the tumours had reached the weight of about 70 gm. X-ray irradiation was then started.

Injections of tissue homogenates were started 13 days after the injections, with the idea of observing the effect of the homogenates in the tumours: 5 groups, each consisting of 10 rats bearing Walker carcinosarcoma, received injection treatment. The rats of one group were given 4 mg. of dried Walker carcinosarcoma, suspended in 1 cc. of saline, by intraperitoneal injection; the second group received the corresponding amount of irradiated Walker tumour, the third an equal amount of dried Yoshida sarcoma, and the fourth an equal amount of irradiated Yoshida sarcoma. The rats of the fifth group were injected with 1 cc. of saline solution daily. Injections were continued until the experimental animals died. Specimens for histological examination were removed before the start of the injection period, and from 30 per cent of the tumors after the death of the animals.

In the second experimental series, injections were begun as early as two days after inoculation. The specimens for histological examination were taken 11 days after inoculation. In the different groups, the number of rats observed for at least three weeks and showing histological evidence of malignant change was as follows: Non-irradiated Walker 7, irradiated Walker 9, non-irradiated Yoshida 4, irradiated Yoshida 4, saline solution 6; total 30 rats.

The growth of the tumours was followed by measurements every second day. The animals were weighed at the same time.

RESULTS

When the injections of tissue homogenates were started 13 days after inoculation, the weight of the experimental animals already showed a decreasing tendency. The size of the tumours then varied from 25 to 60 cu.cm. and their growth was similar in the different groups. During the injection period it was rapid, and no differences were observed between the different groups. The weights of the animals decreased evenly during injection treatment. The rats died three to five weeks after inoculation of tumour, and the average survival time in the different groups did not differ.

The post-mortem histological specimens showed typical tumour tissue, and there were no differences between the tumours of the variously treated animals.

In the series in which injections were started two days after inoculation, the growth tendency of the tumours was essentially similar, though growth was slightly slower during the first two weeks in the groups given tissue homogenates. After this, tumour development in all these groups was completely similar to that in the control group.

No differences were observed as regards the development of the weight and the average survival of the animals in the various groups: the rats survived three to five weeks after inoculation. However, the group treated with Yoshida sarcoma included one histologically malignant infected tumour which, after increasing in size for three weeks to 3 cu.cm., began to diminish in size and disappeared.

All the histological specimens taken from the tumours after death revealed malignant tissue. Necrotic areas, however, were more frequent in the specimens from rats given tissue homogenates. But the tumours in the four groups given different homogenates were not found to differ.

CONCLUSIONS

The Walker carcinosarcoma used in this study is highly malignant. Disappearance of the tumour was only observed in one rat. No differences were observed in the growth rate of the tumours or in the survival time whether the rats had received heterologous or homologous tissue.

It seems obvious that the tumour regression, caused by intraperitoneal injections of homologous tissue in ITB tumour in previous experiments (4) and observed also by Shear & Perrault with sarcoma 37 (2), cannot be generalized. In view of the fact that the whole mechanism of action is unknown, it is difficult to compare experiments made with different tumours. In addition, tissue autolysis and necrosis, depending upon their degree, play a part in the stimulation or inhibition of tissue (1, 4, 9), and so it is also difficult to compare experimental series in which the same tissue has been used: the difference in degree in each case may be deciside. As observations have shown that the amount is of decisive importance (1) and the degree of necrosis is difficult to appraise even morphologically, it is evident that the results must vary considerably.

A proof that growth inhibition occurs is the fact that necrosis and cytolysis are of somewhat higher degree in the tumours of the animals injected with tumour homogenates. A fact in favour of a possible unspecificity of the growth-inhibiting factor is that the necrosis and cytolysis in the specimens from animals injected over a longer period are entirely similar in the groups treated with homologous and with heterologous tissue. Such an unspecificity is also supported by the disappearance of the tumour in one rat injected with Yoshida sarcoma.

It has been demonstrated that, when one of two tumours transplanted into the same rat is irradiated, the non-irradiated tumour also shows a reduction in mitotic activity and retardation of growth (7, 8). On this basis, roentgen irradiation has been assumed to affect the growth-regulating factors. However, as before (4), irradiation of the tumour tissue to be injected was not found in this study to exert any effect on its growth regulating properties.

SUMMARY

1. Rats were inoculated subcutaneously with Walker carcinosarcoma and Yoshida sarcoma. Some of the tumours were X-ray irradiated. The tumours were excised, dried in the refrigerator, and powdered. Homogenates prepared from the powder were injected intraperitoneally into rats which had been inoculated

subcutaneously with Walker carcinosarcoma 2 days or 13 days previously.

2. Neither homologous tissue (homogenates of Walker carcinosarcoma) nor heterologous Yoshida tissue, irradiated or non-irradiated, was found to effect the growth rate of the tumours, the survival of the experimental rats after inoculation, or the weights of the rats — as compared with the controls injected with saline solution. All the experimental animals with histological evidence of uninfected tumour died 3—5 weeks after tumour inoculation.

3. The tumours of those rats in which injections were started 2 days after inoculation, showed a slight necrotizing cytolytic effect. It was similar with all the tissue homogenates used irrespective of wheather they were homologous or heterologous, irradiated or non-irradiated.

REFERENCES

1. GASPARI, W.: Arch. klin. chir., 1927:*146:*711.
2. SHEAR, M. J., and PERRAULT, A.: Proc. Am. Ass. Cancer Res. 1953:*1:*50.
3. TEIR, H.: Growth, 1952:*16:*85.
4. TEIR, H., and VOUTILAINEN, A.: Acta path et microbiol. Scandinav. 1957:*60:*273.
5. WEISS, P.: Yale J. Biol. Med. 1947:*19:*235.
6. WEISS, P.: Science, 1952:*115:*487.
7. VOUTILAINEN, A.: Acta radiol. 1957:*47:*149.
8. VOUTILAINEN, A., and HOPSU, V. K.: Ann. Med. Exper. Fenn. 1957: *35:*198.
9. VOUTILAINEN, A., HOPSU, V. K., and TEIR, H.: Acta path. et microbiol Scandinav. in press.

AUS DEN FORSCHUNGSLABORATORIEN DES STAATLICHEN ALKOHOLMONOPOLS
UND DEM CENTRALLABOR DES KIVELÄ KRANKENHAUSES, HELSINKI

EXPERIMENTELLE STUDIEN ÜBER DIE BIOLOGISCHE WIRKUNG VON ALKOHOL

1. ALKOHOL UND BLUTZUCKER

von

O. FORSANDER, K. O. VARTIA und F-E. KRUSIUS

(Der Schriftleitung eingegangen am 22 Juli 1958

Allgemein bekannt ist, dass Alkoholverbrauch einen Einfluss auf den Blutzuckerspiegel haben kann. Jedoch sind bei Versuchen, die sowohl mit Menschen als auch mit Versuchstieren angestellt wurden, recht unterschiedliche Ergebnisse erhalten worden und die Auffassungen darüber, in welcher Richtung der Blutzucker beeinflusst wird, schwanken. Während ein Teil der Forscher zu dem Resultat kommt, dass Alkohol den Blutzucker senkt (2, 3, 9, 24, 26, 30) konstatieren andere eine Erhöhung (4, 11, 12, 17). Noch mehr Arbeiten scheinen aber darauf hinzudeuten, dass der Einfluss verschieden sein kann und von den vorliegenden Versuchsbedingungen abhängt (5, 6, 13, 14, 16, 21, 25, 27, 30). Bei Zuckerkranken hat man eine Senkung des Blutzuckers durch Alkohol festgestellt oder aber war garkeine Wirkung zu beobachten. Bei chronischen Alkoholikern ist nach Auffassung vieler Forscher der Kohlenhydratumsatz gestört und findet man oft, auch ohne Alkoholverabreichung, erniedrigte Nüchternwerte (10, 18, 21).

Tannent (30) hat in Tierversuchen feststellen können, dass eine Erhöhung des Blutzuckers nach Alkoholverabreichung nur eintritt, falls die Glykogenspeicher der Leber gefüllt sind. Werden die Tiere hingegen vor dem Versuche Hunger ausgesetzt dann tritt keine

Veränderung im Blutzuckerspiegel ein, da der Glykogenvorat aufgebraucht ist. Es wird also anscheined Blutzucker nach Alkoholbelastung durch Spaltung des Leberglykogens mobilisiert. Überhaupt wird die Ansicht vertreten, dass die Oxydation des Alkohols auf Kosten des Leberglykogens vorsichgeht (5, 6, 7, 16, 23, 28, 30). Matunga (7) hat auch in systematischen Versuchen mit Kaninchen gezeigt, das Alkohol durch Entleeren der Leber-Glykogenspeicher hyperglykämisch wirkt. Alkohol ruft diese Wirkung durch Splanichusreizung hervor, durchschneidet man die Nervenfasern bleibt sie aus.

Versuche, welche die ebenfalls beschriebene Senkung des Blutzuckers nach Alkoholgenuss erklären könnten, sind bisher nicht angestellt worden. Pansini (30) nimmt an, dass die Blutzuckersenkung auf eine Hemmung der Zuckerabsorption zurückzuführen ist oder auch durch den Alkoholumsatz selbst hervorgerufen wird. Nizulescu (24) glaubt wiederum, dass Alkohol die Insulinsekretion befördert.

Die vorliegende Arbeit, die zu einer Untersuchung über den Einfluss von Alkoholbelastung auf verschiedene Stoffwechselvorgänge gehört, beschäftigt sich mit der Frage, wie Alkohol, verabreicht in verschiedenen Portionen, auf den Blutzuckerspiegel wirkt.

VERSUCHSANORDNUNG UND MATERIAL

Der Blutalkoholgehalt wurde zu verschiedenen Zeitpunkten nach einer einmaligen Alkoholgabe, nach zwei getrennten Dosen von 1½ Stunden Zwischenraum und ferner nach einer mässigen Alkoholdose, die 5—10 Stunden nach sehr reichlichem Alkoholmissbrauch verabreicht wurde, bestimmt. Für die beiden zuerst erwähnten Versuchsreihen wurden nur Personen gewählt, die seltenen und mässigen Alkoholverbrauch angaben. Zu dem dritten Belastungsversuch wurden Personen gewählt, die nach starkem Alkoholgenuss am vorhergehenden Abend von der Polizei wegen Trunkenheit angehalten worden waren. Es handelte sich nicht um chronische Säufer, nur einer war schon früher wegen des gleichen Vergehens mit der Ordnungsmacht in Konflikt gekommen. — Die Versuche wurden morgens auf nüchternen Magen vorgenommen. Alle Versuchspersonen, die entweder Alkohol nicht vertrugen und während des Versuches über Übelsein klagten oder durch die Blut-

entnahme stärker beeinflusst wurden, sind bei der Bewertung der Ergebnisse nicht berücksichtigt worden. Der Alkohol wurde in Form von 44 G/V% Whisky, verdünnt mit Wasser, verabreicht und innerhalb von einer halben Stunde getrunken. Der Whisky enthielt keinen Zucker. Die Blutalkoholbestimmungen wurden entweder nach Widmark (33) oder unter Anwendung der Mikrodiffusionsmethode von Newman und Newman (22) ausgeführt. Der Blutzuckergehalt wurde kolorimetrisch mittels des Verfahrens von Somogyi-Nelson (20, 29) ermittelt, welches nur »wirklichen» Zucker gibt. Die Blutproben für die Alkoholbestimmungen wurden der Armvene entnommen, der Blutzucker wurde dagegen im Kapillarblut bestimmt.

Am ersten Versuch nahmen insgesammt 10 Personen teil. Alle bekamen 150 ml Whisky, welches durchschnittlich 0.85 g reinem Alkohol pro kg Körpergewicht entspricht. Ein Kontrollversuch ohne Alkoholbelastung wurde mit denselben Versuchspersonen während einer gleichlangen Versuchsperiode angestellt. Am zweiten Versuch beteiligten sich 6 Personen. Sie erhielten Alkohol in zwei Portionen, erst 150 ml Whisky und 1½ Stunden später weitere 75 ml. Die dritte Versuchsreihe bestand aus 16 Personen. Sie erhielten, als sie am Morgen von ihrem Rausch erwachten, 50 ml Whisky, also durchschnittlich 0.3 g reinen Alkohol pro kg Körpergewicht, und eine Stunde später wurde der Blutalkohol- und Zuckergehalt wieder ermittelt. Drei Tage später wurde denselben Personen morgens auf nüchternen Magen und ohne vorhergehenden Alkoholgenuss noch eine Kontrollblutprobe entnommen.

ERGEBNISSE

In Abb. 1 ist die prozentuelle Veränderung der Blutzuckerwerte zu den verschiedenen Zeitpunkten nach der Belastung im Vergleich zum Nüchternwert im eigentlichen Versuch und im Kontrollversuch graphisch dargestellt. Auch die durchschnittliche Blutalkoholkurve ist der Übersichthalber angegeben. In der ersten Versuchsreihe nimmt der Blutzucker zuerst deutlich zu und erreicht sein Maximum nach einer halben Stunde. Hiernach sinkt der Blutzucker wieder und zwar viel stärker als im Kontrollversuch, in welchem die Abnahme während der Beobachtungszeit gleichmässig aber verhältnismässig gering ist. Die Erhöhung des Blutzucker-

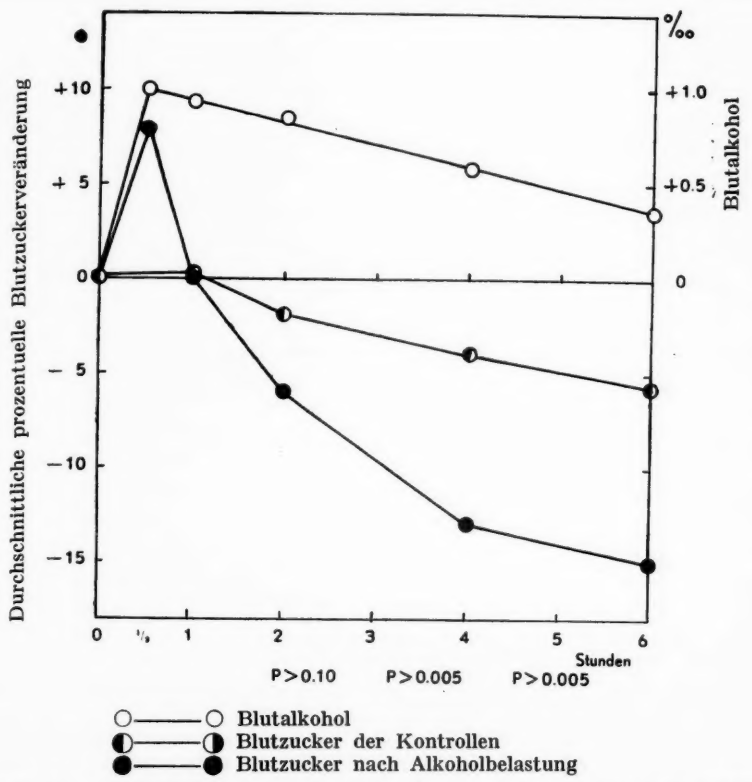


Abb. 1. — Durchschnittlicher Blutalkoholgehalt und durchschnittliche Veränderung der Blutzuckerkonzentration in Prozent des Nüchternwertes von 10 Personen nach einmaliger Belastung mit 150 ml 44 G/V% Alkohol und bei einem entsprechenden Kontrollversuch ohne Alkohol.

spiegels ist im Alkoholversuch nach einer halben Stunde recht beträchtlich (8%). Andererseits ist die Senkung des Blutzuckers 4—6 Stunden nach der Belastung signifikativ grösser als im Kontrollversuch ($P > 0.005$). Der Alkohol wurde nach der Belastung schnell absorbiert, die Alkoholkonzentration im Blut erreichte ihr Maximum bereits nach einer halben Stunde. Danach war die Abnahme derselben linear, aber noch nach 6 Stunden war der Blutalkoholgehalt deutlich erhöht.

In der zweiten Versuchsreihe (Abb. 2), in welcher 2 Alkoholgaben gegeben wurden, stieg der Blutzucker wie in der ersten Serie und der höchste Wert wurde nach einer halben Stunde erreicht. Die zweite Alkoholdose verursachte eine neue Steigerung, die aber

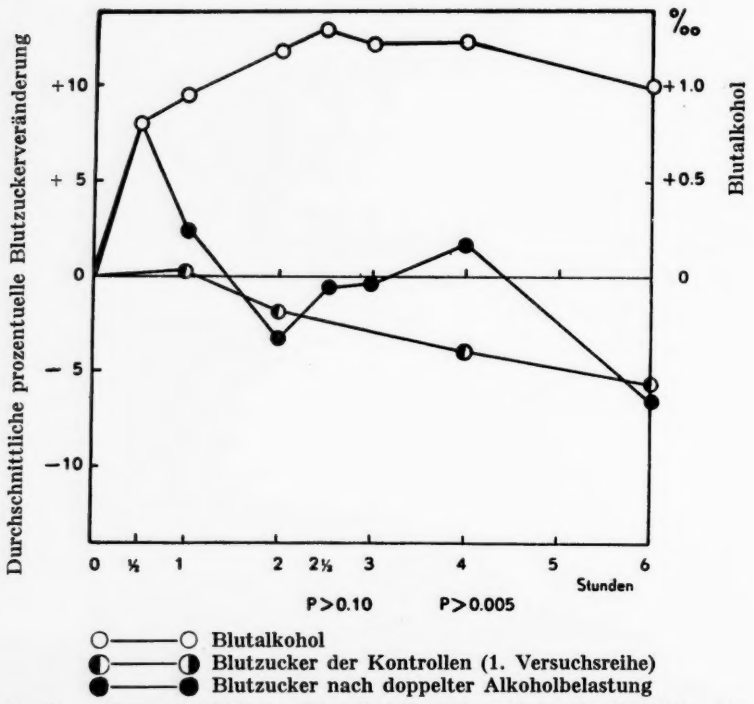


Abb. 2. — Durchschnittlicher Blutalkoholgehalt und durchschnittliche Veränderung der Blutzuckerkonzentration in Prozent des Nüchternwertes von 6 Personen nach zweimaliger Belastung mit 150 bzw. 75 ml 44 G/V % Alkohol mit 1 ½ Stunden Zwischenraum. Zum Vergleich sind die Blutzuckerveränderungen des Kontrollversuches der ersten Versuchsreihe eingezeichnet.

niedriger war und langsamer eintrat. Der Höhepunkt wurde jetzt erst nach 2 Stunden erreicht, wonach der Blutzuckerspiegel absank. Verglichen mit dem Kontrollversuch der ersten Versuchsreihe waren beide Blutzuckersteigerungen sehr deutlich. Der Blutalkoholgehalt stieg bei diesen Versuchspersonen etwas langsamer als im ersten Versuch und das Maximum nach der ersten Alkoholgabe lag zwischen 1—1½ Stunden.

Der Blutalkoholgehalt der Personen der dritten Versuchsreihe war zu Beginn des Versuches sehr variierend (Tab. 1). Auch der Blutzuckergehalt schwankte ziemlich stark, war aber durchschnittlich verhältnismässig niedrig. Nach der Whiskybelastung am Morgen nach dem Rausch stieg sowohl die Blutzucker- als auch Alkoholkonzentration im Blut. Der Kontroll-Blutzuckerwert drei Tage später ohne Alkoholgenuss war aber durchschnittlich höher.

TABELLE 1

BLUTZUCKER- UND ALKOHOLKONZENTRATION AM MORGEN NACH SCHWEREM RAUSCH UND EINE STUNDE SPÄTER NACH WEITERER BELASTUNG MIT 50 ML 44 G/V % ALKOHOL NEBST KONTROLLWERTEN OHNE VORHERGEHENDEN ALKOHOLGENUSS

Nr.	Blutzucker mg %			Blutalkohol ‰		Bemerkungen
	Nach Alkoholgenuss		Kontrollwerte	0 St.	1 St.	
	0 St.	1 St.				
1	62	67	116	0.19	0.86	
2	85	100	—	1.40	1.52	
3	67	76	88	0.21	0.67	
4	100	107	—	0.63	0.90	
5	69	76	—	1.32	1.75	
6	79	79	84	0.86	1.40	
7	94	98	98	0.92	1.31	
8	74	74	100	1.23	1.59	
9	87	96	86	1.06	1.30	
10	87	44	90	0.48	0.65	
11	74	83	87	1.76	1.98	
12	72	81	83	1.30	1.99	
13	76	84	88	1.04	1.33	Allgemeinbefinden sehr schlecht
14	44	60	—	1.14	1.25	» » »
15	61	43	91	2.31	2.40	Schwindel, Kollaps
16	94	116	78	1.73	1.95	Übelsein
M	76.7	80.3	90.8			

DISKUSSION

Die Ergebnisse zeigen, dass Alkohol einen Einfluss auf den Blutzuckerspiegel hat. Zuerst wird der Blutzucker durch Alkoholgenuss erhöht, später wird er signifikativ gesenkt. Diese Wirkung trat in allen Fällen ein. Die Tatsache, dass man früher bei Untersuchungen über den Einfluss von Alkohol auf den Blutzucker so wechselnde Ergebnisse erzielt hat lässt sich vielleicht dadurch erklären, dass die Blutzuckerbestimmungen zu verschiedenen Zeitpunkten ausgeführt wurden.

Die Steigerung des Blutzuckers in unseren Versuchen stimmt gut überein mit denjenigen früheren Untersuchungen, die gezeigt haben, dass Alkohol das Leberglykogen mobilisiert und dadurch den Blutzucker erhöht. Beim Menschen scheint diese Wirkung

schon nach viel kleineren Alkoholgaben zum Vorschein zu kommen als im Tierversuch. Bei Tieren erhielt man einen hypoglykämischen Effekt erst nach Verabreichung von mehr als 2.4 g Alkohol pro kg Körpergewicht. Der Versuch mit der doppelten Alkoholbelastung bekräftigt die Annahme von Matunga (16), dass der Alkohol nicht direkt auf die Leber wirkt sondern über das sympathische Nervensystem. Eine erneute Alkoholgabe steigerte den Blutzucker nochmals beträchtlich, obwohl der Blutalkoholgehalt schon im Voraus erhöht war und erst später noch weiter antieg. Auch Köhlers Resultat (14) passt hierzu, denn er erhielt keinen Effekt auf den Blutzucker, wenn der Alkohol intravenös gegeben wurde. Die Blutzucker-steigernde Wirkung des Alkohols nach starkem Alkoholmissbrauch verbunden mit Hypoglykämie, ist wahrscheinlich eine der Wirkungen, die man unbewusst erstrebt mit einem Alkoholtrunk während der Katerperiode.

Es ist nicht ohneweiteres klar warum der Alkohol im späteren Stadium nach der Belastung den Blutzucker senkt. Aber, wenn man gewisse Parallelen zum Umsatz der Fettsäuren zieht, gibt sich eine Erklärung, die wahrscheinlich erscheint. Nach Oxydation zu aktiviertem Acetat verläuft sowohl die weitere Verbrennung der Fettsäuren als auch des Alkohols über dasselbe Enzymsystem. Man hat auch gezeigt, dass Fütterung mit Fett bei Versuchstieren den Blutzucker senken kann (1). Wenn man Versuchstieren grössere Mengen Ketonkörper einspritzt nehmen die Glykogenspeicher der Leber ab und Hypoglykämie kann eintreten (19, 31, 32). Da der Alkohol in der Leber zu Essigsäure abgebaut wird und in dieser Form oder als Ketonkörper extrahepatisch weiter oxydiert wird (8, 15), ist es denkbar, dass nicht der Alkohol selbst sondern seine Abbauprodukte die Senkung des Blutzuckers verursachen.

ZUSAMMENFASSUNG

Der Einfluss von Alkohol auf den Blutzuckerspiegel wurde untersucht. Anfangs steigert Alkoholgenuss den Blutzucker, aber später tritt eine Senkung ein. Die initiale, steigernde Wirkung sieht man auch nach wiederholter Alkoholbelastung, kurze Zeit nach der ersten Dose, oder 5—10 Stunden nach schwerem Alkoholmissbrauch.

LITERATURVERZEICHNIS

1. Bickenbach, W. & Junkersdorf, P.: Arch. exp. Path. Pharmakol. 1928:*132:*129.
2. Blatherwick, N. R., Maxwell, L. C. & Long, M. L.: Amer. J. Physiol. 1924:*67:*346.
3. Burdi, I.: Z. ges. exp. Med. 1930:*71:*480.
4. Edkins, N. &. Murray, M. M.: J. Physiol. 1931:*71:*403.
5. Eger, W. & Ottensmeier, H.: Med. Monatschr. Stuttgart, 1954:*8:*85.
6. Forbes, J. C. & Duncan, G. M.: Quart. J. Stud. Alc. 1950:*11:*373.
7. Forsander, O.: Suom. Kemistil. 1957:*B 30:*99.
8. Forsander, O., Räihä, N. & Suomalainen, H.: Z. physiol. Chem., Hoppe-Seyler 1959:*312:*1.
9. Gavrila, G. & Sparches, T.: C. R. Soc. Biol. 1928:*98:*65.
10. Glatt, M. M.: Brit. Med. J. 1955:*1:*973.
11. Hetenyi, G.: Z. ges. exp. Med. 1924:*40:*261.
12. Himwich, H. E., Nachum, L. H., Rakieten, N., Fazekas, J. F., DuBois, D. & Gildea, E. F.: J. Amer. Med. Assoc. 1933:*100:*651.
13. Kanai, I.: Biochem. Z. 1933:*262:*41.
14. Koehler, A. E., Hill, E. & Buttenwieser, E.: Fed. Proc. 1944:*3:*59.
15. Lundsgaard, E.: C. R. Lab. Carlsberg 1938:*22:*333.
16. Matunaga, H.: Tohoku J. exp. Med. 1942:*44:*130.
17. Mauro, V.: Rass. Med. industr. 1942:*13:*254.
18. Möllerström, J.: Ark. Kemi Min. Geol. 1945:*19:A:*26.
19. Nath, M. C. & Chakrbarti, C. H.: Proc. Soc. exp. Biol. Med. 1954: *86:*851.
20. Nelson, N.: J. Biol. Chem. 1944:*153:*375.
21. Neves, D. P., Faria, C. V. & Fujioka, T.: Rev. Hosp. Clin. S. Paulo 1950:*5:*115.
22. Newman, E. J. & Newman, H. W.: Stanford Med. Bull. 1953:*11:*96.
23. Newman, H. W., Van Wikle, W., Kennedy, N. K. & Morton, M. C.: J. Pharmacol. Exp. Therap. 1940:*68:*194.
24. Nitzulescu, J., Zosin, C. & Ionescu, M.: Rév. med.-chir., Paris 1948: *59:*68.
25. Oppermann, F.: Dtsch. Z. Nervenhk. 1913:*47—48:*590.
26. Pansini, R. & Casaula, A.: Omnia med. 1952:*30:*93.
27. Pansini, R. & Casaula, A.: Boll. soc. ital. Biol. sper. 1951:*27:*910.
28. Salant, W.: J. Biol. Chem. 1907:*3:*403.
29. Somogyi, M.: J. Biol. Chem. 1945:*160:*62.
30. Tannent, D. M.: Quart. J. Stud. Alc. 1941:*2:*263.
31. Tidwell, H. C. & Axelrod, H. E.: J. Biol. Chem. 1948:*172:*179.
32. Tidwell, H. C. & Nagle, M. E.: J. Biol. Chem. 1953:*201:*727.
33. Widmark, E. M. P.: Die theoretischen Grundlagen und die praktische Verwendbarkeit der gerichtlich-medizinischen Alkoholbestimmung, Berlin 1932.

AUS DEM CENTRALLABOR DES KIVELÄ KRANKENHAUSES UND DEN FORSCHUNGSLABORATORIEN DES STAATLICHEN ALKOHOLMONOPOLS, HELSINKI

EXPERIMENTELLE STUDIEN ÜBER DIE BIOLOGISCHE WIRKUNG VON ALKOHOL

2. ALKOHOL UND NEBENNIERENRINDEN-FUNKTION

von

F-E. KRUSIUS, K. O. VARTIA und O. FORSANDER

(Der Schriftleitung eingegangen am 22. Juli 1958)

Chronischer Alkoholismus kann bekanntlich toxische Veränderungen in den Nebennieren hervorrufen. Smith (20) stellte schon 1951 fest, dass ACTH- und Adrenalininjektionen bei Alkoholikern eine geringere Abnahme der eosinophilen Leukozyten im peripheren Blut hervorriefen als bei Kontrollpersonen. Dieselbe Beobachtung machten auch Dowden und Bradbury (2) bei Adrenalineinspritzungen. Andererseits ist aber in aller letzter Zeit wiederholt hervorgehoben worden, dass man nicht berechtigt ist aus dem Eosinophilensturz direkte Schlussfolgerungen über die Funktion der Nebennierenrinde anzustellen.

Lowell und Tintera (11) konstatierten bei Alkoholikern oft Hypoglykämie und verminderte 17-Ketosteroidausscheidung im Harn. Mårtens (13) berichtet ebenfalls über vorübergehende Abnahme der 17-Ketosteroidausscheidung im akuten Stadium von chronischem Alkoholismus. ACTH und Cortison wird auch ziemlich allgemein zur Behandlung von chronischem Alkoholismus empfohlen (1, 10, 21, 23).

Die Wirkung akuter Alkoholvergiftung auf die Nebennierenfunktion ist auch experimentell untersucht worden. Kinzius (6) fand, dass absoluter Alkohol innerhalb von 15—30 Minuten nach

Genuss eine Erhöhung der Blutadrenalinwerte hervorruft. Später trat jedoch eine Senkung ein, die umso deutlicher war je mehr Alkohol verabreicht wurde. Laves (9) verfolgte die Zahl der eosinophilen weissen Blutkörperchen im peripheren Blut nach Verabreichung von Weinbrand und beobachtete durchschnittlich eine Steigerung, die bei Asthenikern stärker war als bei Pyknikern. Die letzteren brauchten verhältnismässig mehr Alkohol, damit die nach Cortisonverabreichung auftretende Eosinopenie verhindert werden konnte. Binswanger (1) konstatierte wiederum bei Versuchspersonen, die durch starken Weingenuss »offensichtlich vergiftet» waren, eine Abnahme der eosinophilen Leukozyten während der ersten 90 Minuten, der dann eine Vermehrung folgte, die eine Stunde dauerte. Hiernach nahm die Zahl der Eosinophilen wieder ab.

In zahlreichen Tierversuchen hat man nach Verabreichung grosser Alkoholmengen Stress-Symptome festgestellt. Smith (22) zeigte, dass Alkoholfütterung den Askorbinsäure- und Cholesterolgehalt der Nebennieren-Rindenschicht senkt. Dieser Effekt blieb aber bei hypophysectomierten Tieren aus. Forbes und Duncan (3) konnten diese Ergebnisse in grossen Versuchsreihen mit Meerschweinchen und Ratten bestätigen. Sie fanden, dass gleichzeitig mit der Abnahme der Askorbinsäure und des Cholesterols in den Nebennieren der Gehalt derselben in den Leberparenchymzellen zunimmt. ACTH-Verabreichung ruft dieselben Veränderungen hervor aber in noch kürzeren Zeit. Gillissen (5) fand in Tierversuchen nach grossen Dosen von Weisswein Vergrösserung der Zellkerne der Zona fasciculata. Diese Veränderung wurde durch reinen Alkohol nicht hervorgerufen. Kliewe und Gillissen (7) berichteten auch, dass Weissweinverabreichung bei Ratten eine Zunahme der 17-Ketosteroidausscheidung verursache, während reiner Alkohol diesen Effekt nicht habe. Santisteban und Swinyard (18) untersuchten bei Mäusen den Einfluss von Alkohol auf die Nebennierenfunktion, wobei sie das Gewicht der Thymus als Massstab benutzten. In ihren Versuchen, in denen die Mäuse 3 g Äthylalkohol per kg Körpergewicht intraperitoneal eingespritzt bekamen, steigerte der Alkohol die Nebennierenfunktion 25—50-fach.

Zusammenfassend kann man also feststellen, dass chronischer Alkoholgenuss anscheinend degenerative Veränderungen der Nebennieren hervorruft. Sehr hohe, einmalige Alkoholgaben führen bei Versuchstieren zu auf Stress hinweisenden Symptomen. Dasselbe

Ergebnis hat man aber bei Versuchspersonen nicht erzielt, wohl vor allem, weil viel kleinere Dosen verwendet werden müssen als in Tierversuchen. Auch die verabreichte Alkoholart und die Konstitution der Versuchspersonen scheint Einfluss zu haben auf die Versuchsergebnisse.

Der Zweck dieser Untersuchung war an Hand von Plasma 17,21-Dihydroxy-20-ketosteroidbestimmungen (17—OHCS) und von Zählung der eosinophilen Blutkörperchen Einblick zu gewinnen in die Wirkung von Äthylalkohol auf die Funktion der Nebennieren-Rindenschicht.

VERSUCHSANORDNUNG UND MATERIAL

Die Versuche wurden mit denselben Personen vorgenommen wie in unserer Arbeit über den Einfluss von Alkoholverbrauch auf den Blutzucker (4). Die Versuchsanordnung war ebenfalls die Gleiche. Die Blutproben für die Plasma 17-OHCS-Bestimmungen wurde der Armbeugevene entnommen und in mit Heparin versetzte Zentrifugengläser überführt. Die Proben wurden morgens auf nüchternen Magen vor Belastung mit Alkohol und ½, 1, 2, 4 bzw. 6 Stunden danach genommen. Die Zählung der eosinophilen Blutkörperchen wurde in Kapillarblut, welches zu denselben Zeitpunkten entnommen wurde, ausgeführt. In der ersten Versuchsreihe, in der einmal 150 ml 44 G/V% Whisky, verdünnt mit Wasser, verabreicht wurde, waren insgesammt 10 Versuchspersonen. In der zweiten Versuchsreihe mit doppelter Alkoholbelastung, bei der 150 ml + 75 ml Whisky mit 1½ Stunden Zwischenraum verabreicht wurden, waren nur 5 Personen. Wegen der geringen Zahl der Versuchspersonen wurde der Mittelwert dieser Serie nicht berechnet. Die dritte Versuchsreihe, zu der Personen am Morgen 5—10 Stunden nach schwerem Rausch gewählt wurden, umfasste 16 Personen. Chronische Alkoholiker waren nicht in dem Material. Nach Entnahme der »Nüchternblutprobe» erhielten diese Personen 50 ml 44 G/V% Whisky und 1 Stunde später wurde eine zweite Blutprobe entnommen. Nach 3 Tagen wurde ferner noch eine Kontrollblutprobe am Morgen auf nüchternen Magen und ohne vorhergehenden Alkoholgenuss untersucht.

Als Kontrollen für die erste und zweite Versuchsreihe wurde 12 Personen beider Serien verwendet, deren Plasma 17-OHCS-

Gehalt ebensowie die Zahl der Eosinophilen 2—3 Wochen später unter exakt denselben Versuchsbedingungen und zu denselben Zeitpunkten, aber ohne Alkoholgenuss, bestimmt wurden.

Die Plasma 17-OHCS-Bestimmungen wurden nach der Silber-Porter-Methode (19) in der Modifikation von Peterson, Karrer und Guerra (16) ausgeführt. Die Zählung der eosinophilen Blutkörperchen wurde in der Zählkammer nach Färbung mit Phloxin in 50% Propylenglykokollösung vorgenommen (17). Zur Kontrolle und zur Berechnung des prozentuellen Gehalts an Eosinophilen wurde natürlich auch die Gesamtzahl der weissen Blutkörperchen gezählt.

ERGEBNISSE

Die Plasma-17-OHCS-Werte der ersten und zweiten Versuchsreihe sind in Tab. 1 wiedergegeben, die der Versuchsreihe 3 in Tab. 2. Die 17-OHCS-Werte des Nüchternplasmas variierten bei allen Ver-

TABELLE 1

PLASMA 17-OHCS-WERTE NACH EINFACHER ALKOHOLBELASTUNG (150 ML 44 G/V% WHISKY) UND DOPPELTER ALKOHOLBELASTUNG (150 ML + 75 ML WHISKY MIT 1½ STUNDEN ZWISCHENRAUM) NEBST WERTEN DER KONTROLLSERIE, OHNE ALKOHOLBELASTUNG

Nr.	Nach Alkoholbelastung Stunden						Ohne Alkoholbelastung Stunden				
	0	½	1	2	4	6	0	1	2	4	6
	1. Versuchsreihe										
1.	21	14	9	9	7	18	4	3	3	10	4
2.	15	9	9	17	13	6	8	6	7	12	5
3.	12	10	8	7	10	7	8	7	3	7	9
4.	13	19	20	12	8	10	13	13	7	7	8
5.	15	8	8	10	11	9	11	10	9	11	11
6.	18	18	15	9	7	12	10	15	11	—	13
7.	18	10	13	13	12	14	13	8	7	9	12
8.	7	4	7	4	5	6	7	7	7	10	20
9.	10	11	9	7	6	15	16	12	9	15	10
10.	10	17	12	8	9	11	14	11	8	12	8
M_1	13.9	12.0	11.0	9.6	8.8	10.8					
	2. Versuchsreihe										
11.	14	16	13	10	17	13	13	9	10	14	12
12.	18	14	15	8	10	9	12	7	7	6	3
13.	6	4	—	5	3	8					
14.	12	9	8	17	10	7					
15.	15	10	4	6	5	9	M_K 10.8	9.1	7.3	10.0	9.6

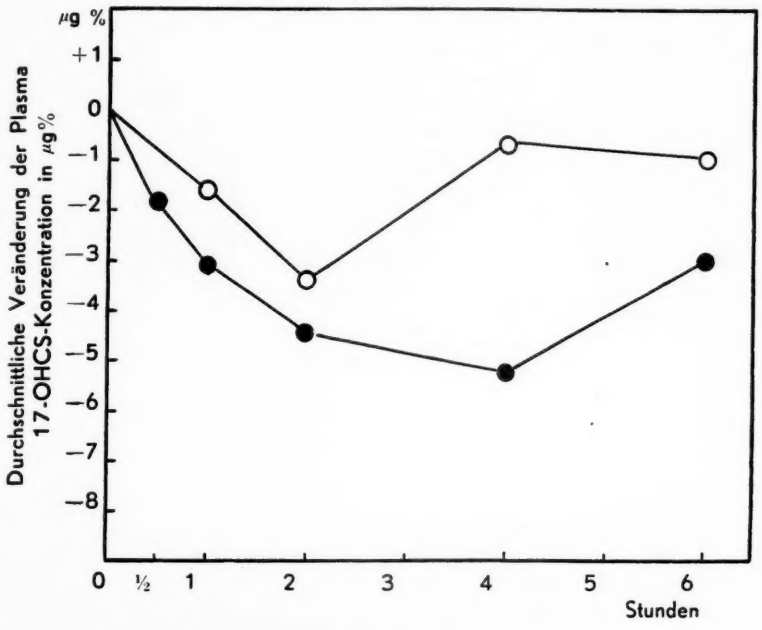
TABELLE 2

PLASMA 17-OHCS-KONZENTRATION AM MORGEN NACH SCHWEREM RAUSCH UND EINE STUNDE SPÄTER NACH WEITERER BELASTUNG MIT 50 ML 44 G/V % ALKOHOL NEBST KONTROLLWERTEN OHNE VORHERGEHENDEN ALKOHOLGENUSS

Nr.	Plasma 17-OHCS μg%			Bemerkungen
	Nach Alkoholgenuss		Kontroll-werte	
	0 St.	1 St.		
1	14	23	22	
2	14	16	—	
3	24	18	14	
4	25	14	—	
5	11	19	—	
6	29	28	28	
7	18	17	21	
8	28	38	24	
9	22	29	18	
10	34	49*	24	*Hypoglykämie
11	23	18	19	
12*	29	17	41	
13	30	32	20	Allgemeinbefinden sehr schlecht
14	38*	45	—	*Hypoglykämie + » »
15	13	23*	17	* » + Schwindel, Kollaps
16	18	21	16	Übelsein
M	22.7	26.0	20.3	

* Nr. 12 nicht berücksichtigt bei Berechnung des Mittelwertes wegen des unnormal hohen Kontrollwertes.

suchspersonen der beiden ersten Serien und bei den Kontrollen innerhalb normaler Grenzen. Der Mittelwert von 27 Bestimmungen betrug 12.3 μg% mit Grenzwerten von 4—21μ g%. Dieses Ergebnis stimmt gut überein mit früheren Untersuchungen aus diesem Laboratorium (8, 14). Jedoch hatten die Versuchspersonen vor der Alkoholbelastung durchschnittlich etwas höhere Nüchternwerte als vor den Kontrollversuchen. In Versuchsreihe 1 betrug der Mittelwert 13.9 μg% gegenüber 10.8 μg% bei den Kontrollen. Sowohl nach Alkoholbelastung als auch ohne Alkoholbelastung trat bei den Versuchspersonen im Verlauf von 6 Stunden durchschnittlich eine geringe Abnahme der Plasma 17-OHCS-Werte auf. Die niedrigsten Werte wurden bei der ersten Versuchsreihe nach 2—4 Stunden und bei den Kontrollen nach 2 Stunden konstatiert (Abb. 1). Die Abnahme der Plasma-17-OHCS-Werte nach Ver-



○——○ Plasma 17-OHCS des Kontrollversuches
●——● Plasma 17-OHCS nach Alkoholbelastung

Abb. 1. — Durchschnittliche Veränderung der Plasma 17-OHCS-Konzentration in µg% von 10 Personen nach einmaliger Belastung mit 150 ml 44 G/V% Alkohol und bei einem entsprechenden Kontrollversuch ohne Alkohol.

abreichung von 150 ml Whisky war durchschnittlich höher als bei den Kontrollen, ein statistisch signifikativer Unterschied bestand aber nicht.

Nach Alkoholbelastung trat in der ersten Versuchsreihe ein Eosinophilensturz ein, der durchschnittlich bis 50% betrug (Abb. 2). Die Abnahme war 2 Stunden nach der Alkoholbelastung am grössten, aber auch nach 4 Stunden war sie noch deutlich. In der Kontrollgruppe war keine sichere Veränderung in der prozentuellen Zahl der Eosinophilen während der Versuchsperiode zu konstatieren. Die prozentuelle Abnahme der Eosinophilen in der Versuchsreihe ist nicht durch eine Zunahme der Gesamtleukozytenzahl zu erklären, denn diese verbleibt in der Versuchsreihe verhältnismässig konstant, während die Leukozytenzahl der Kontrollserie durchschnittlich bis zu 30% ansteigt. Da die individuellen Eosino-

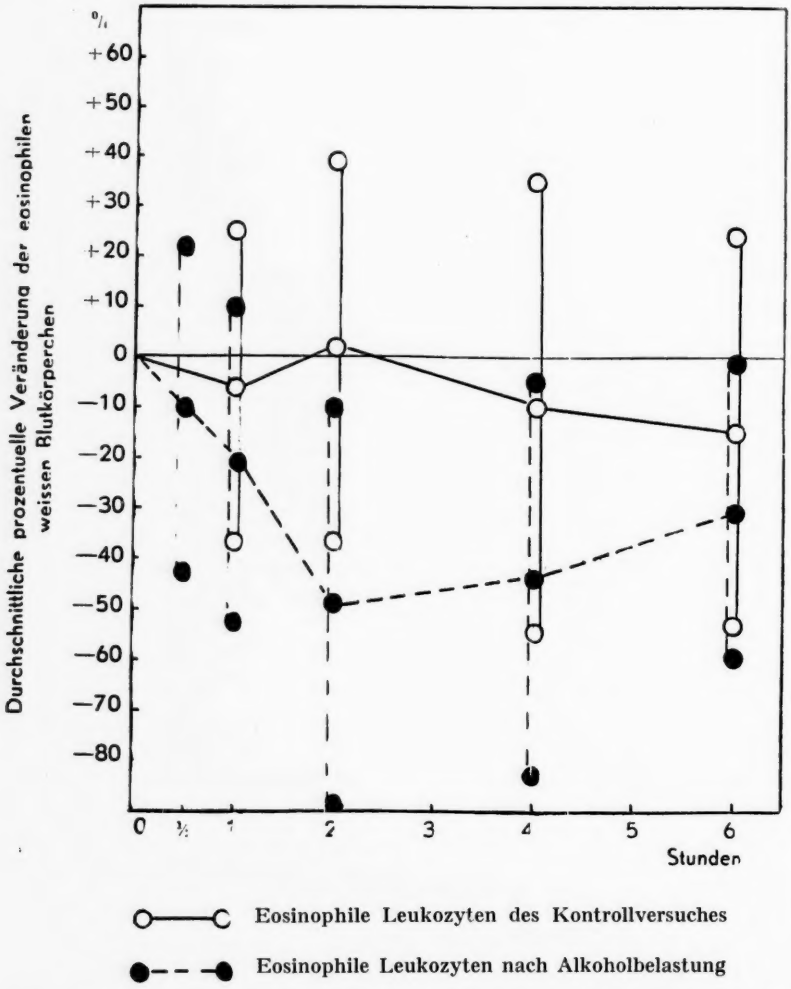


Abb. 2. — Durchschnittliche Veränderung der eosinophilen Leukozyten in Prozent des Nüchternwertes von 10 Personen nach einmaliger Belastung mit 150 ml 44 G/V % Alkohol und bei einem entsprechendem Kontrollversuch ohne Alkohol.

philenwerte jedoch stark schwankten wurde davon abgesehen dieses kleine Material statistisch zu verwerten.

Bei den Rauschpersonen der dritten Versuchsreihe (Tab. 2) war der Plasma 17-OHCS-Durchschnittswert am Morgen vor der erneuten Alkoholbelastung schon deutlich erhöht mit 22.7 μg%

und Grenzwerten von 11—38 μg%. Auch der Kontrollwert drei Tage später ohne vorhergehenden Alkoholgenuss war bei einigen Fällen noch erhöht, der Durchschnittswert betrug 20.3 μg% mit Grenzwerten von 14—28 μg%. Ein Fall (Nr. 12) hatte einen Kontrollwert von 41 μg%. Da bei diesem Fall der 1-Stundenwert nach Alkoholgenuss normal war, ist er bei der Berechnung des Mittelwertes nicht berücksichtigt worden. 1 Stunde nach Belastung mit 50 ml Whisky stieg der durchschnittliche Plasma-17-OHCS-Wert auf 26 μg%, mit Grenzwerten von 14—49 μg%. Die Fälle 10, 14 und 15 hatten Hypoglykämie mit Blutzuckerwerten von 44,44 resp. 43 mg% (4). Diese Fälle hatten auch mit die höchsten Plasma-17-OHCS-Werte.

BESPRECHUNG

In Übereinstimmung mit den Versuchen von Binswanger (1) konnten wir 2 Stunden nach Belastung mit 150 ml Whisky einen deutlichen und starken Eosinophilensturz feststellen. Dieses Ergebnis wird meistens als Zeichen einer gesteigerten Nebennierenrinden-Aktivität bewertet. Jedoch zeigen unsere gleichzeitig ausgeführten Plasma 17-OHCS-Bestimmungen, dass die Sekretion Cortisolartiger Substanzen ins Blut nicht vermehrt sondern eher vermindert ist. Eine gesteigerte Nebennierenrindenfunktion kann also nicht die Ursache der Eosinopenie sein, die übrigens zeitlich auch früher eintritt als nach Verabreichung von ACTH- bzw. Cortison. Eine Erklärung derselben scheint uns an Hand unserer Versuche zur Zeit nicht möglich. Die Diskrepanz zwischen dem Plasma 17-OHCS-Spiegel und der Zahl der Eosinophilen zeigt aber, dass ein Eosinophilensturz von 50% nicht immer und ohneweiteres als spezifische Äusserung einer gesteigerten Nebennieren-Aktivität gedeutet werden darf.

Die Abnahme der Plasma 17-OHCS-Konzentration nach Belastung mit 150 ml Whisky war im Vergleich zu den Werten des Kontrollversuches nicht statistisch signifikativ. Ein ähnliches Ergebnis ergab ebenfalls die kleine Versuchsserie mit doppelter, mässiger Alkoholbelastung. Aus früheren Untersuchungen (12, 15) wissen wir, dass der Plasma 17-OHCS-Gehalt morgens auf nüchternen Magen am höchsten ist und im Verlauf des Tages sinkt.

Dieselbe Erscheinung war in unserem Material in der Kontrollserie zu konstatieren. Da unsere Serien aber verhältnismässig klein sind und die Unterschiede zwischen der ersten Versuchsserie und den Kontrollen nicht beweisend sind, muss hervorgehoben werden, dass Alkoholbelastungen mit Dosen von 150—225 ml 44 G/V% Whisky, entsprechend 0.3—0.45 g Alkohol per kg Körpergewicht, keinen sicheren Einfluss auf die Plasma 17-OHCS-Konzentration haben und somit auch die Nebennieren-Funktion nicht beeinflussen.

In der Rauschserie ist der Einfluss des Alkohols auf die Plasma 17-OHCS-Konzentration auch nicht eindeutig. Es scheint jedoch, dass das Niveau der Plasma 17-OHCS-Werte 5—10 Stunden nach schwerem Alkoholmissbrauch, also im Rausch, höher als normal ist. Der Unterschied im Vergleich zu unseren normalen Nüchternplasma-Werten ist deutlich. Wiederholte Belastung mit einer kleinen Portion Alkohol scheint die Hormonkonzentration im Blut noch weiter zu erhöhen. Der hohe Durchschnittswert wird jedoch durch einige sehr hohe Einzelwerte bedingt, die auf eine deutliche Hyperreaktivität der Nebennierenrinde hinweisen. Sonderbarerweise hatten diese Versuchspersonen auch niedrige Blutzuckerwerte, einige sogar deutliche Hypoglykämie. Ebenso war in diesen Fällen die Blutalkoholkonzentration relativ hoch (4). Klinisch waren Übelsein, schlechtes Allgemeinbefinden sowie Schwindel und Kollapssymptome zu beobachten. Obwohl in diesen Fällen eine deutliche Hyperfunktion der Nebennierenrindenschicht, beurteilt auf Grund der hohen Plasma 17-OHCS-Werte, vorlag, war die Reaktion bei den übrigen Versuchspersonen nicht so sicher oder deutlich. In gewissem Masse sprechen jedoch die Ergebnisse der Rauschserie dafür, dass extreme Belastung mit Alkohol auch beim Menschen zu einer Hyperfunktion der Nebennierenrinde und zu Stress-Symptomen führen kann. Dieses Resultat ist in Einklang mit den früher referierten Ergebnissen in Tierversuchen (3, 22).

ZUSAMMENFASSUNG

Der Einfluss von Alkohol auf die Funktion der Nebennierenrinde wurde untersucht. Als Kriterien wurden Veränderungen des Plasma 17-OHCS-Spiegels und der Eosinophilenkonzentration im Kapillarblut verwendet. Nach einmaliger Belastung mit 150 ml 44

G/V% Whisky, verdünnt mit Wasser, war im Vergleich zur Kontrollserie 2—4 Stunden nach der Belastung eine Abnahme der Plasma 17-OHCS-Konzentration festzustellen, der Unterschied war aber nicht statistisch signifikativ. Ein durchschnittlicher Eosinophilensturz von 50% wurde konstatiert. Das Ergebnis bei doppelter Belastung mit 150 + 75 ml Whisky mit 1½ Stunden Zwischenraum war ähnlich, die Serie aber zu klein um mit Sicherheit bewertet werden zu können. In Versuchen mit Personen in schwerem Rausch wurden 5—10 Stunden nach extremen Alkoholmissbrauch durchschnittlich erhöhte Plasma 17-OHCS-Werte konstatiert, die eine Stunde nach weiterer Belastung mit 50 ml Whisky noch anstiegen.

Die Versuche zeigen, dass einmalige und mässige Belastung mit Alkohol keinen sicheren Einfluss auf die Nebennieren-Funktion, beurteilt an Hand von Plasma 17-OHCS-Bestimmungen, hat. Der auftretende Eosinophilensturz kann nicht als spezifisches Zeichen einer Funktionssteigerung der Nebennierenrinde gedeutet werden. Extreme Alkoholbelastung scheint aber innerhalb von 5—10 Stunden zu einer Steigerung der Nebennieren-Funktion führen zu können.

LITERATURVERZEICHNIS

1. Binswanger, H.: Schweiz. Med. Wchnschr. 1952:*82:*513.
2. Dowden, C. W. & Bradbury, J. T.: J. Amer. Med. Assoc. 1952:*149:* 725.
3. Forbes, J. C. & Duncan, G. M.: Quart. J. Stud. Alc. 1951:*12:*355; 1953:*14:*19, 22.
4. Forsander, O., Vartia, K. O. & Krusius, F-E.: Ann. Med. exp. & Biol. Fenn. 1958:*36:*412.
5. Gillissen, G. Hyg. 1952:*135:*341.
6. Kinzius, H.: Arbeitsphysiologie 1950:*14:*243.
7. Kliewe, H. & Gillissen, G.: Experientia 1955:*11:*237.
8. Krusius, F-E., Oka, M. & Kärkelä A.: Scand. J. Clin. & Lab. Invest. 1958:*10:*323.
9. Laves, W.: Arch. exp. Path. Pharm. 1954:*22:*482.
10. Lowell, H. W. & Tintera, J. W.: Geriatrics 1949:*4:*274.
11. Lowell, H. W. & Tintera, J. W.: Alcoholism 1952:*80:* .
12. Mach, R. S.: Ciba-Symposium 1956:*4:*116.
13. Mårtens, S.: Nord. Med. 1953:*49:*466.
14. Oka, M.: Acta Med. Scandinav. 1956:*156:*221.
15. Pekkarinen, A.: Persönliche Mitteilung, 1958.

16. PETERSON, R. E., KARRER, A. & GUERRA, S. L.: Anal. Chem. 1957:*29:* 144.
17. RANDOLPH, T. G.: J. Allergy 1944:*15:*89.
18. SANTISTEBAN, G. A. & SWINYARD, C. A.: Endocrinology 1956:*59:*391.
19. SILBER, R. H. & PORTER, C. C.: J. Biol. Chem. 1954:*210:*923.
20. SMITH, J. J.: Proc. 2nd clin. ACTH-Conf. 1951:*2:*161.
21. SMITH, J. J.: New York State J. Med. 1950:*50:*1704; J. Amer. Med. Assoc. 1953:*152:*384.
22. SMITH, J. J.: J. clin. Endocrinol. 1951:*11:*792.
23. VOEGTLIN, W. L.: Quart. J. Stud. Alc. 1953:*14:*28.

FROM THE DEPARTMENT OF PATHOLOGY, SECTION II, UNIVERSITY OF HELSINKI

EXPERIMENTAL ANALYSIS OF CERTAIN CRITERIA OF TISSUE GROWTH[1]

by

JUHANI RAPOLA, LAURI SAXÉN and HARALD TEIR

(Received for publication July 23, 1958)

The purpose of the present study was to assess certain current routine methods from the standpoint of the demonstration of tissue growth and to define their usefulness and significance. The methods in question are used in investigations concerning the stimulation and inhibition of the cellular growth of various organs. Furthermore, the question was posed whether it is necessary to use more than one of these methods in studies of tissue growth, or one method alone.

It was premised that two fresh pieces of tissue are to be investigated, namely, a normal, not growing control specimen and a piece of tissue suspected of accelerated growth. In such cases it is not possible to use the most accurate procedures involving the determination *in vivo* or *in vitro* of the metabolism and other functions of the growing tissue. Methods requiring pretreatment of the sample *in vivo*, such as the isotope technique, can also be left out of consideration. In practice, the situation often resembles the one here described, and then the number of appropriate methods is limited. In this laboratory calculation of mitoses, determination of the nucleic acids and measurement of nuclear size have been employed for the purpose in question.

As experimental material, regenerating liver tissue obtained by partial hepatectomy was chosen. This tissue regenerates rapidly

[1] This investigation was supported by the Sigrid Jusélius Foundation and the Damon Runyon Memorial Fund (DRG—291—C).

and regarding it a large body of data is available. On the basis of previous investigations we know the growth rate (8), the variations in mitotic frequency (6, 5), the variations in concentration of RNA and DNA (7, 5), and the changes in nuclear size (3, 11, 6). Since most of these investigations are based on extensive series and their results are almost consistent, our intention was not to analyse the available data in detail. Our object was to present comparative results on the accuracy and usefulness of the different methods.

MATERIAL AND METHODS

Sprague-Dawley male rats, weighing from 250—350 g., were used. Prior to and during the experiments they were kept under identical conditions and on the same diet.

Two different experiments were performed.

Experiment I. — On 16 rats a vertical incision, about 3 cm. long, was made under ether anaesthesia through the abdominal layers beginning from the proc.xiphoideus. From the liver thus exposed the two median lobes were ligated with silk and removed. The portion thus removed constituted about 30 per cent of the total weight of the liver. This sample was used as control material. None of the animals died from the operation. The animals were killed 24, 48 and 96 hours after operation. At sacrifice, the liver was in many cases variegated, and many rats exhibited clearly necrotic lobes. No determinations were made on macroscopically or microscopically necrotic lobes.

Experiment II. — On 15 rats partial hepatectomy was performed by the original method of Higgins & Andersson, *i.e.* by ligation and removal of the two median lobes and the left lateral lobe, together forming an anatomic entity which can readily be removed (8). The portion thus removed constituted about 65—70 per cent of the total weight of the liver. None of the animals died from the operation. The animals were killed 48 hours, 7 days and 14 days after operation. They exhibited a light, evenly coloured liver surface; no necrotic lobes were observed macroscopically or microscopically. Simultaneously with the last experimental group, 5 rats were sacrificed as controls.

The tissues were fixed for 24 hours in Bouin's solution, mounted in paraffin after a dehydration series and sectioned at 4 μ. The

sections were stained by Mayer's hemalum-eosin method. For the determination of nuclear size, serial sections at 8 μ were made from three livers of each group. These sections were stained by Feulgen's nuclear reaction.

The mitoses were counted from 100 visual fields at 10 $\times$ 30 magnification, the area of one visual field being 0.24 mm^2. The number of mitoses has been indicated per 1,000 liver cells. Cell density was determined by counting the cells of 25 visual fields from each preparation, using 10 $\times$ 100 magnification and oil immersion (the area of the visual field being 0.02 mm^2).

The nuclei were drawn through a camera lucida at 2200 $\times$ magnification. On these drawings the size of the nuclei was measured with a planimeter. Three preparations from each experimental group in exp. I were investigated, 500 nuclei being measured for each preparation.

From all the samples mentioned in the material the RNA and DNA were determined by the methods of Schneider (10) and Ogur-Rosen (9). In addition, in exp. II the nucleic acids were determined from 10 liver lobes removed in the hepatectomy.

RESULTS

The results are presented in Tables 1 and 2.

The results of the nucleic acid determinations performed on the liver lobes removed in exp. II were practically identical with those obtained on the control material, namely, RNA 3.90 $\pm$ 0.10 and DNA 0.98 $\pm$ 0.26.

In order to facilitate evaluation of the significance of the results with regard to the growth process at various stages, graphical representations of the results are in Text-figures 1 and 2 compared with graphs showing the significance of the results as calculated by Student's t-test.

At the stage of most intensive growth in exp. I, RNA, DNA and the rate of mitosis all proved to be good criteria of growth (Text-fig. 1), though differing from each other in that RNA reached its maximum first, the rate of mitosis then, and DNA as the last.

The determinations presented in Text-fig. 2 were made at intensive, slow and very slow stages of growth, respectively. After 70 per cent hepatectomy the liver had regenerated in 48 hours to 50

per cent of its original weight, in 7 days to about 74 per cent, and after 14 days only some 7 per cent were lacking of the original weight, which is attained about 14—20 days after operation (8). It is seen in the figure that the rate of mitosis and RNA were both markedly increased ($P < 0.0001$), whereas DNA did not exhibit any significant increase after 48 hours. After 7 days, the values for RNA still showed a significatioant elevation, whilst the increase in the rate of mitosis was no longer significant. At 14 days after operation none of the methods employed yielded significant results.

Summing up, it may be stated that in the present investigation RNA proved to be by far the best criterion of growth at all stages. The rate of mitosis seems to be a satisfactory criterion when growth is intensive. With regard to DNA, the results obtained in the different experiments were contradictory, and the determination of nuclear size did not yield significant results.

In what follows, these components will be analysed in detail.

As emphasized above, RNA proved to be the best criterion of growth in the two experiments described in the foregoing. In practice, however, the significance of this method is limited by the fact that the concentration of RNA is very sensitive to any metabolic changes in the tissue. The RNA concentration in the liver is dependent e.g. on the nutritional conditions (4). Thus, RNA is an indicator of the metabolic function of the tissue more than it is a specific criterion of tissue growth (2).

Our results on DNA were contradictory. In exp. I it showed a highly significant increase, but in exp. II it exhibited no significant change. It seems obvious that if a sufficiently large material is available, DNA is a reliable criterion of accelerated growth. In exp. I our control material was large enough to enable the demonstration of a significant increase in DNA at the stage of rapid growth. In exp. II, on the other hand, we intentionally used a control material which was not larger than the experimental groups. Owing to the scantiness of the control material, no significant increase in DNA could be demonstrated. It seems to be of some importance to emphasize the general fact that where quantitative changes in a biological material are the object of study, reliable results cannot be obtained unless the quantity used for comparison is highly stable. In our exp. II, for instance, a reliable result could only have been obtained if the control material had been larger, and the

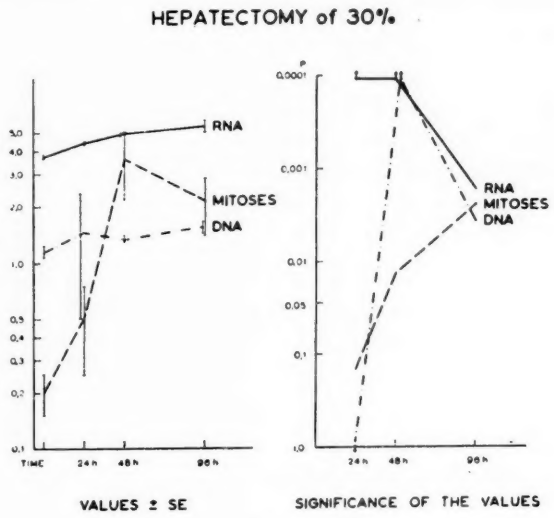

HEPATECTOMY of 30%
RNA
MITOSES
DNA
TIME
24 h
48 h
96 h
VALUES ± SE
SIGNIFICANCE OF THE VALUES

Fig. 1.

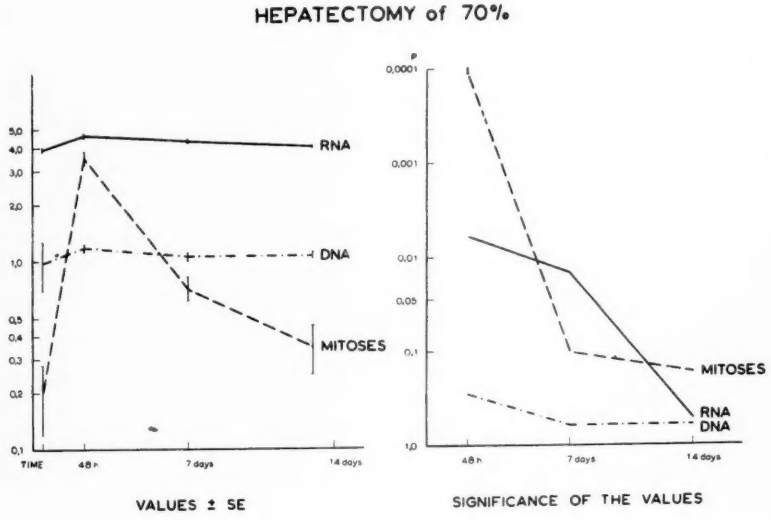

HEPATECTOMY of 70%
RNA
DNA
MITOSES
TIME
48 h
7 days
14 days
VALUES ± SE
SIGNIFICANCE OF THE VALUES

Fig. 2.

TABLE 1

THE RESULTS OF EXPERIMENT I. (RNA AND DNA AS PERCENTAGES OF THE DRY WEIGHT, MITOSES PER 1000 CELLS AND THE RELATIVE SIZE OF LIVER NUCLEI). TIME IN HOURS AFTER 30 PER CENT HEPATECTOMY

	Control		24 h.		48 h.		96 h.	
	No. of Rats	Mean ± SE	No. of Rats	Mean ± SE	No. of Rats	Mean ± SE	No. of Rats	Mean ± SE
RNA	16	3.71 ± 0.094	4	4.41 ± 0.091	5	4.90 ± 0.070	5	5.43 ± 0.474
DNA	16	1.13 ± 0.082	5	1.46 ± 0.914	5	1.35 ± 0.050	5	1.55 ± 0.114
Mitoses	16	0.2 ± 0.057	2	0.5 ± 0.245	5	3.6 ± 1.340	5	2.15 ± 0.670
Nuclear size	3	1.28 ± 0.396	3	1.52 ± 0.058	3	1.64 ± 0.122	3	1.73 ± 0.071

TABLE 2

RESULTS OF EXPERIMENTS 2. (RNA AND DNA AS PERCEETAGES OF DRY WEIGHT, MITOSES PER 1000 CELLS AND THE NUMBER OF CELLS IN ONE VISUAL FIELD, 0.0 2MM²). TIME IN HOURS AFPER 65 PER CENT HEPATECTOMY

	Control		48 h.		168 h.		336 h.	
	No. of Rats	Mean ± SE	No. of Rats	Mean ± SE	No. of Rats	Mean ± SE	No. of Rats	Mean ± SE
RNA	4	3.91 ± 0.11	5	4.51 ± 0.129	5	4.35 ± 0.100	5	3.96 ± 0.100
DNA	4	0.98 ± 0.29	5	1.19 ± 0.041	5	1.05 ± 0.049	5	1.07 ± 0.042
Mitoses	5	0.2 ± 0.080	5	3.6 ± 0.273	5	0.72 ± 0.090	5	0.35 ± 0.122
Number of cells	5	5.2 ± 0.17	5	5.4 ± 0.32	5	5.0 ± 0.5	5	4.7 ± 0.22

mean error thus had been smaller. Furthermore, it was found in exp. I that DNA reacted slowly, a significant increase being demonstrable only 48 hours after the beginning of the experiment, whereas RNA showed a marked increase after 24 hours. In any case it seems obvious, if the changes observed in exp. II are at all worthy of consideration, that DNA is an inadequate indicator of slow growth, considering that by the seventh day it had already dropped almost to the level of the control.

It is obvious that an increased rate of mitosis is an expression of accelerated growth. Increases in the rate of mitosis which are marked enough to be measurable are, of short duration, however, persisting for only some 2—3 days, and individual variations are great. It may, thus, be assumed that in different individuals the rate of mitosis reacts with different promptness, the maximum rate being attained at different points of time after operation. Since, moreover, the duration of a mitosis is only a few hours (13), it is readily understood that the results obtained on a scanty material show a wide dispersion, which lessens the value of this method as an indicator of growth. In addition, it has been observed that fluctuations occur in the rate of mitosis (5).

In our experiments nuclear size did not increase significantly, and not even the small mean increase which occurred can be regarded as a specific indicator of tissue growth, considering that, for instance, intensified function of a glandular tissue is accompanied by a marked increase in size of the cells (»funktionelles Kernoedem», 1) without any tissue growth proper being involved. In addition, Sulkin (12) has observed that the number of nuclear classes do not change in regenerating liver tissue. It has also been observed that after hepatectomy the fluid concentration in the tissues increases (6, 15), causing a swelling of the cells and the nuclei which cannot in general be regarded as any specific sign of growth. The fact that the determination of nuclear size is time consuming even to the experienced is a further reason for rejecting this method in routine studies of growth.

It should be added that, according to our observations, the changes in cell density are so inconsiderable that the mitoses could just as well have been counted directly from the visual fields without determining the cell frequency.

On the basis of the results presented in the foregoing it appears

that when slow growth or slight growth stimulation are the object of study, the use of more specific methods is indicated, considering that the methods here discussed would in such cases require very large materials.

SUMMARY AND CONCLUSION

Two experiments were performed, both involving partial hepatectomy in male rats. In experiment I, 30 per cent of the liver was removed, in experiment II 70 per cent. After operation, RNA, DNA and the rate of mitosis were determined in the regenerating liver. In exp. I, the determinations were performed 24, 48 and 96 hours after operation, in exp. II after 48 hours, 7 days and 14 days. In addition, in exp. I nuclear size was measured in three individuals from each experimental group.

The results show, firstly, that the methods in question are inadequate when the purpose is to show that a tissue is not growing. Seven days after hepatectomy the liver tissue is still undergoing a slow growth (8), but this could not be demonstrated by the methods and material used in the present experiments. Furthermore, it appears that determination of the nucleic acids alone is not sufficient for the demonstration of tissue growth. The rate of mitosis is obviously the most adequate criterion of these three, but in a small material significant changes are demonstrable by this method only if the tissue investigated is in a stage of rapid growth. If the tissue growth is slight, the counting of mitoses can, of coarse, be used as criteria, but the number of experimental animals and the total area of determinations must increase with declining growth rate. During slow growth the number of visual fields necessary for determination, is to large for making the method easy to use.

Where high values for the rate of mitosis, DNA and RNA are obtained, it seems, however, justifiable to conclude that the tissue is rapidly growing, and where high RNA values alone are obtained, it may be concluded that the tissue grows, if changes in metabolism, etc., can be ruled out.

REFERENCES

1. BENNINGHOFF, A.: Paper Read at the Congress of Anatomy, Bonn. 1949.
2. BRACHET, J.: In: CHARGAFF-DAVIDSON: The Nucleic Acids, vol. II: 475—519. Academic Press Inc. New York. 1955.
3. BRUES, A. M., and MARBLE, B. B.: J. Exp. Med. 1937:*65:*15.
4. CAMPBELL, M., and KOSTERLITZ, H. W.: 1.st. Internat. Congr. Biochem. Abstr. Sect. 1. 1949:*24—25:*372.
5. CATER, D. B., HOLMES, B. E., and MEE, L. K.: Acta radiol. 1956:*46:* 655.
6. HARKNESS, R. D.: J. Physiol. 1952:*117:*267.
7. HECHT, L. I., and POTTER, V. R.: Cancer Research. 1956:*16:*988.
8. HIGGINS, G. M., and ANDERSSON, R. M.: Arch. Path. 1931:*12:*186.
9. OGUR, M. and ROSEN, G.: Arch. Biochem. 1950:*25:*262.
10. SCHNEIDER, W. C.: J. Biol. Chem. 1945:*161:*293.
11. STOWEL, R. E.: Arch. Path. 1948:*46:*164.
12. SULKIN, N. M.: Am. J. Anat. 1943:*73:*107.
13. TEIR, H.: Acta Path. et microbiol. Scandinav. 1948:*25:*45.
14. TEIR, H., LARMO, A. L., ALHO, A., and BLOMQVIST, K.: Exp. Cell. Research. 1957:*13:*147.
15. ZAKI, F. G.: Zeitschr. f. Naturforsch. 1954:*9b:*293.

FROM THE INSTITUTE OF FORENSIC MEDICINE, UNIVERSITY OF HELSINKI

DETECTION OF METHANOL POISONING

WITH SPECIAL CONSIDERATION OF THE ESTIMATION OF FORMIC ACID IN SOLID VISCERA, BLOOD AND URINE; INVESTIGATION OF 11 FATAL CASES

by

ANTTI R. ALHA, JYRKI RAEKALLIO, and ANNA-LIISA MUKULA

(Received for publication July 29, 1958)

At this Institute a large number of fatal cases of methanol poisoning are investigated every year. As is well known, the demonstration of increased concentration of formic acid in organ specimens is a significant criterion of methanol poisoning. In this connection the urine has been the subject of intensive study. By contrast, relatively few investigations have been performed on solid viscera and blood (4, 9, 12). We hitherto determined the formic acid concentration in the urine alone, using the method of Autenrieth (3). Since samples of urine are not always available, however, it was regarded as indicated to investigate whether routine detection of methanol poisoning could be based on estimation of the formic acid concentration in various organs and in the blood.

For this purpose brain, kidney, liver, blood and urine were subjected to parallel investigations. The estimation of formic acid was performed by Klauer's (9) modification of Fincke's (8) method, which involves separation by steam distillation and gravimetric determination of the mercurous chloride reduced from mercuric chloride by formic acid. In connection with the isolation, some experiments were made using Bastrup's (5) methyl ester technique. In addition, the length of the reduction time necessary for a quantitative result was investigated, and a study of the quantity of the yield was performed by addition of a known amount of formic acid

to the control specimen. Furthermore, the »normal» concentration of formic acid in the organs under study was determined.

After that 10 cases of acute methanol poisoning (Cases 1—10) were investigated.

In addition, 1 case of acute ethyl alcohol — methyl alcohol poisoning (Case 11) was investigated.

METHODS

The determination of formic acid was throughout performed on 50 g of solid viscera, blood or urine. The urine was distilled as such, whilst 50 g of water were added to the minced organs and to the blood. Before distillation the samples were saturated with sodium chloride (20 g NaCl/50 g sample) as suggested by Lund (11). According to the literature, it is sufficient if the volume to be distilled is 5—10 times the volume of the distillate. In preliminary experiments involving the addition of various amounts of formic acid to the specimens it was found, however, that the yield was far from being quantitative when a volume which was 10 times that of the sample was distilled. In subsequent experiments about 50 mg of formic acid (Merck, pro anal., 98—100 per cent) were added to 50 g of urine, and 20- to 40-fold volumes were distilled. The yield was found to increase by only a few per cent. Therefore, we decided to use 10-fold volumes for distillation and to regard yields of the order of magnitude indicated in what follows as quantitative. The average distillation time was 1 hr/500 ml of distillate, except in the experiments with blood and liver, where the rate of distillation had to be lowered owing to foaming.

In the separation of formic acid Bastrup (5) employed a method based on distillation of the readily evaporating methyl ester of formic acid. In experiments with this technique we were able to confirm Bastrup's contention that it is suitable for the isolation of formic acid from urine. On the other hand when we tried the method in question on a control kidney, which had a »formic acid concentration» of 1.6 mg/50 g as determined by distillation in steam, the corresponding value obtained was 22.6 mg/50 g. Hence an additional, comparative investigation was performed, using 50 g of the same kidney in each experiment. The results of this investigation are shown in Table 1.

TABLE 1

THE »FORMIC ACID CONCENTRATION» IN 50 G OF A CONTROL KIDNEY AS DETERMINED BY DIFFERENT METHODS OF DISTILLATION

Method of Distillation	Volume of Distillate, ml	»Formic Acid», (mg)
1. Distillation in steam	1200	0.50
2. Ethyl ester	150	0.65
3. Methyl ester	150	30.90

Furthermore, on direct determination of formic acid after the addition of 150 ml methanol (Merck, pro anal.) a value corresponding to 1.4 mg formic acid was obtained. This result shows that when methanol is heated together with kidney, a volatile substance reducing mercuric chloride is formed in abundance.

It may be mentioned that Dakin, Janney and Wakeman (7) and Riesser (13) have used a technique suitable for accurate quantitative investigations which involves the extraction of formic acid.

In order to estimate the length of the reduction time required after addition of mercuric chloride, an experiment was performed which involved direct determination in aqueous solutions to which about 25 mg of formic acid had been added. The results appear in Table 2.

TABLE 2

REDUCTION TIME AND YIELD OF FORMIC ACID

Reduction Time (hrs.)	Yield, %
1	89.8
2	93.3
3	98.4
4	99.6

In the present investigation a reduction time of 3¼ hours was used.

In order to estimate the quantitaty of the yield with the method of formic acid determination used, organ material from subjects killed in accidents was used in experiments in which 48.8 mg of formic acid were added to the samples. The results are shown in Table 3 (in calculating the results, the values obtained on control determinations without the addition of formic acid are taken into account).

TABLE 3

THE YIELD OF ADDED FORMIC ACID

Sample Investigated	Yield in % of Formic Acid Added
3 samples of urine	78.3—86.4
7 » » brain.....	70.2—93.2
6 » » kidney	77.0—89.6
2 » » liver	65.9—73.1
2 » » blood	26.6—36.6

It is seen that the yield was by no means quantitative. The best results were obtained with urine, brain and kidney. The difference in results obtained with the same organs from different subjects are in part attributable to variations in quality of the samples, *e.g.* state of decomposition and lipid concentration, which are reflected, in particular, in the readiness or the degree of difficulty by which distillation in steam is performed.

The »normal» concentration of formic acid in the samples to be investigated was determined not only in connection with the experiment just described, but also by investigation of the urine of more 8 subjects killed in accidents. The results are shown in Table 4.

TABLE 4

»NORMAL» FORMIC ACID CONCENTRATION, MG/100 G

Sample Investigated	»Formic Acid»
11 samples of urine	0.24—5.02
7 » » brain.....	0.62—3.40
6 » » kidney	0.02—2.08
2 » » liver	0.50—5.08
2 » » blood	0.46—1.96

The highest value for the »normal» formic acid concentration was thus about 5 mg/100 g.

With regard to the isolation of formic acid from organ specimens by distillation in steam it has been objected (11, 13) that on heating in the presence of an acid, the carbohydrates of the specimens may produce formic acid.

In addition, it may be mentioned that according to Riesser (13), formic acid may be determined by iodometric determination of mercurous chloride, which is an appropriate method in many serial investigations (11).

INVESTIGATION OF FATAL CASES

When the methodological aspects had been clarified, organ specimens from 10 successive fatal cases of acute methanol poisoning were investigated (Cases 1—10). In all cases a qualitative methanol determination was performed on mixed viscera, and an estimation of the formic acid concentration was made on brain, kidney and liver, on blood in 7 cases, and on urine in 8 cases (in 2 cases no urine was available).

In addition, in 5 cases (nos. 6—10) Widmark's reduction value (15) was determined in blood, and determination of ethanol in the blood was performed by the ADH method. Furthermore, quantitative determination of methanol was performed in the blood and the urine by the chromotropic acid technique. The results are shown in Table 5.

Qualitative Methanol Determination on Mixed Viscera. — About 300 g of mixed viscera, slightly acidified with tartaric acid, was distilled in steam to a 50 ml distillate. A qualitative methanol determination was performed on 1 ml of distillate, using the generally known chromotropic acid reaction. On the basis of previous practice we have become used to expressing the strength of the violet colour by the symbols +, ++ and +++. According to previous, rough quantitative estimation, + corresponds to about 0.05—0.10 ‰, ++ to about 0.2—2.0 ‰ and +++ to over 2.0 ‰ methanol in the distillate under investigation.

In addition, formaldehyde was determined by the chromotropic acid reaction in 1 ml of distillate, the results being negative in all cases. Qualitative ethanol reactions were, too, in all cases negative.

Ethanol Determination in Blood by the ADH Method. —This was performed by the method of Bonnichsen and Lundgren (6) as modified at this Institute (2).

Quantitative Methanol Determination in Blood and Urine. — Blood and urine were distilled by the method of Friedemann-Klaas as modified by Weinig (14). The methanol in the distillate was determined by the method of Agner and Belfrage (1) as modified by Koivusalo (10).

Furthermore, the results obtained in a case of ethanol — methanol poisoning may be reported (Case 11).

Jr. no. 258/58. This person had been found dead at a public lavatory. At autopsy, there was a marked odour of household spirits (Finnish household spirits contain some 2—6 per cent methanol in addition to ethanol, dye stuffs and denaturing substances). Steam distillation of mixed viscera yielded some methanol, an abundance of ethanol and, in addition, a small amount of acetaldehyde. On determination by the ADH method the fol-

TABLE 5

RESULTS IN 10 FATAL CASES OF METHANOL POISONING

Case No.	Jr. No.	Qualitative Methanol Determination	Formic acid. mg/100 g					Blood Alcohol, ‰		Quantitative Methanol Determination, ‰	
			Urine	Brain	Kidney	Liver	Blood	Widmark Value	ADH	Blood	Urine
1	43/58	+	257.4	29.3	26.0	28.8	6.5				
2	71/58	+++	134.6	31.0	33.8	21.8	14.0				
3	79/58	++	163.6	34.6	35.8	27.4	17.6				
4	96/58	++		35.9	49.9	41.5	30.6				
5	101/58	+++		34.6	45.0	24.3	17.5				
6	129/58	+++	213.3	35.7	51.8	37.8	13.7	1.30	0.00	0.90	1.43
7	131/58	++	276.1	37.5	35.6	15.0	7.9	1.23	0.00	0.97	1.12
8	136/58	++	305.7	44.1	76.0	45.7		2.09	0.00	1.54	1.36
9	140/58	++	296.9	50.5	70.8	54.5		2.65	0.00	1.96	3.06
10	158/58	++	111.9	44.0	45.5	31.6		0.80	0.20	0.53	0.68

lowing results were obtained: ethanol concentration 4.56 ‰ in the blood and 5.19 ‰ in the urine; methanol concentration about 0.4 ‰ in the blood and 0.5 ‰ in the urine. The concentration of formic acid was 2.3 mg/100 g in the urine and 1.1 mg/100 g in the brain.

DISCUSSION

With regard to the concentration of formic acid, the highest values were obtained in urine and the lowest in blood. It would obviously be interesting to compare the concentration of formic acid in urine, blood and various viscera on the one hand and the results obtained on quantitative methanol determination on the other. Considering, however, the great differences in the quantitaty of the yield, which was revealed by the study of the methodological aspects, it is clear that no confident conclusions can be drawn. On the other hand in all of the 10 cases of acute methanol poisoning investigated, the formic acid concentration in the organ specimens was markedly higher than »normal». In addition, by steam distillation of the samples, methanol was qualitatively demonstrated.

In the special investigations performed in cases 6—10, a slightly elevated ethyl alcohol value was obtained by the ADH method in one case alone, whereas Widmark's value was markedly elevated in all 5 cases. On quantitative methanol determination definitely

increased values were obtained in both the blood and the urine. These special investigations did not throw any additional light on the cases concerned.

It is sometimes necessary to examine cases of methanol poisoning which are so acute that the formic acid concentration of the viscera has not yet risen before death has occurred. In such cases special investigations are, of course, essential, as shown by our case 11.

SUMMARY

The purpose was to ascertain whether in forensic practice it would be possible to use the determination of formic acid in brain, kidney, liver and blood as a criterion of methanol poisoning, if no urine is available.

In the estimation of formic acid concentration, distillation in steam was used for the isolation, and the oxidative reaction with mercuric chloride for the determination, the yield of mercurous chloride being measured gravimetrically.

In the methodological part of the investigation, the quantity of the yieid was the object of study. The yield was found to be of the magnitude 70—90 per cent in both the urine, and the brain and the kidneys. By contrast, the liver and, in particular, the blood gave smaller yields. In addition, the »normal» formic acid concentration in the material was determined, being in all cases 5 mg/100 g or less.

In 10 fatal cases of methanol poisoning the formic acid concentration in the organ specimens was determined by the above-mentioned method, markedly higher values than »normal» being obtained. In all cases methanol was qualitatively demonstrated in steam distillate of mixed viscera.

The present investigation shows that for the detection of acute methanol poisoning it is often sufficient in forensic practice, when methanol has been qualitatively detected in the organism, to prove that the concentration of formic acid is increased in the urine or, if urine is not available, in the viscera, preferably the brain or the kidneys.

REFERENCES

1. Agner, K., and Belfrage, K. E.: Acta physiol. scandinav. 1947:*13:*87.
2. Alha, Antti R., Hjelt, E., and Tamminen, V.: To be published later.
3. Autenrieth, W.: München. med. Wchnschr. 1919:*66:*862.
4. Bannicke, H.: Diss. Halle. Ref. Klauer, H.: Deutsche Ztschr. ges. gerichtl. Med. 1938:*30:*280.
5. Bastrup, J. Th.: Acta pharmacol. et toxicol. 1947:*3:*303.
6. Bonnichsen, R., and Lundgren, G.: Ibid. 1957:*13:*256.
7. Dakin, H. D., Janney, N. W., and Wakeman, A. J.: J. Biol. Chem. 1913:*14:*341.
8. Fincke, H.: Biochem. Ztschr. 1913:*51:*253.
9. Klauer, H.: Deutsche Ztschr. ges. gerichtl. Med. 1938:*30:*280.
10. Koivusalo, M.: Studies on the Metabolism of Methanol and Formaldehyde in the Animal Organism. — Acta physiol. scandinav. 1956: *39:*Suppl. 131.
11. Lund, A.: Acta pharmacol. et. toxicol. 1948:*4:*99.
12. Lund, A.: Ibid. 1948:*4:*205.
13. Riesser, O.: Biochem. Ztschr. 1923:*142:*280.
14. Weinig, E.: Deutsche Ztschr. ges. gerichtil. Med. 1950—51:*40:*318.
15. Widmark, E. M. P.: Die theorethischen Grundlagen und die praktische Verwendbarkeit der gerichtlich-medizinischen Alkoholbestimmung. — Urban & Schwarzenberg, Berlin und Wien, 1932.

FROM THE DEPARTMENT OF FORENSIC MEDICINE AND THE DEPARTMENT OF PATHOLOGY, SECTION II, UNIVERSITY OF HELSINKI

EFFECT OF ACUTE AND PROLONGED HISTAMINE SHOCK ON THE CYTOLOGY OF THE ADRENAL CORTEX

by

L. LÖFGREN and A. KIVIRANTA

(Received for publication July 29, 1958)

The close relationship between the hypophysis and the adrenal cortex is revealed at alarm reaction — caused by numerous kinds of stress — by the increased hormone production in the adrenal cortex. This activity which is caused by ACTH can be studied by establishing the changes in amount and distribution of, for instance, ascorbic acid and lipids by means of various histochemical tests.

After hypophysectomy and the resultant cessation of the ACTH action, the size of the cells in the adrenal cortex is reduced. W. Boguth *et al.* (5) have shown, moreover, that a morphokinetic adaptation reaction is also reflected in the size of nucleus in the fascicular zone after hypophysectomy, and after various kinds of stress causing an alarm reaction which is a stage in Selye's well-known General-Adaptation-Syndrome. In the latter case, the arisal of a morphokinetic adaptation reaction presupposes that the duration of the increased hormonal action of the hypophysis is such that the mentioned adaptation, in the form of structural changes, enlargement of cells, and cellular increase, is required to cause increased functional activity in the adrenal cortex. To judge from the results of W. Boguth's *et al.* investigations, the morphokinetic reaction is revealed by the changes in the nuclear size at a fairly early stage of changed cellular activity in the adrenal cortex. — The cell increase is revealed by the mitotic activity in the so-

called stable and labile organs, as distinguished from the so-called perennial organs (2, 3). The mitotic ratio in adrenals of normal guinea-pigs has been studied, for instance by H. T. Blumenthal (4), and the changes in the mitotic ratio have been investigated, among others, by R. Dallwig (9).

The present study was made for the purpose of finding out, if possible, how the size of the nuclei in the fascicular zone of the cortex react to an acut and prolonged histamine shock . Attention was also paid to the mitotic ratio under these conditions. These studies are connected with a series of earlier investigations regarding the action of histamine shock and prolonged application of histamine on the organism (21, 22, 23, 24).

MATERIAL AND METHODS

The series consisted of 59 3—4 month-old guinea-pigs. All the animals were males which may be of significance as certain differences attributable to sex have been observed in the size of the adrenal glands (1, 13, 14) and some cyclic alterations with, for instance, different mitotic ratio in estrus and diestrus (1, 6). The weight of the majority of the animals was 450—550 grammes, and all were sexually mature, hence no influence on the mitotic ratio due to growth need be considered, as the mitotic activity in animals which have reached puberty is but little according to H. T. Blumenthal (4). The guinea-pigs were kept isolated in a room with a fairly constant temperature, and fed on a diet consisting of hay; fresh grass, oats, and turnips. The tests were made in late spring and summer, and in autumn.

In order to obtain an as even and prolonged histamine action as possible, the histamine dihydrochloride[1] was embedded in a mixture of beeswax and paraffin oil according to the method described by L. J. Hay *et al.* (15) and modified, as reported in a previous paper (22). The animals were given daily intramuscular injections of the mixture in doses corresponding to about 2 mg histamine base for 1, 3, 12 or 16 days. The guinea-pigs were decapitated about six hours after the last injection. The decapitation

[1] Our thanks are due to Messrs. Hoffmann — La Roche & Co., Ltd. for supplying the histamine required for these experiments.

was done in the afternoon or evening, in order to exclude possible diurnal rhythmical variation in size of nucleus, observed in some organs by T. Casperson and Hj. Holmgren (7) and P. Nicolay (25), and in the mitotic rate as reported by H. Klein and H. Geisel (20). — The animals suffering from acute shock were decapitated at once when it became evident that recovery was impossible. — The behaviour of the animals during acute histamine shock has been reported in detail in a previous work (23).

The guinea-pigs were dissected inmediately after decapitation, the adrenal gland was divided by a vertical cut into two equal halves and immersed in Bouin's fluid. Fixation lasted as a rule for 12 hours; the preparation was then embedded in paraffin, cut into 4 μ slices and stained with hemalum-eosin.

The contours of the nuclei in the outer spongiocyte layer of the fascicular zone were outlined with the aid of Abbe's drawing apparatus, a technique used, for instance, by H. Teir (32) and others. The subject and the drawing board were kept on the same level. The enlargement thus obtained, and controlled at intervals by micrometer, was 2500 $\times$. Contours of 200 nuclei per slice were drawn; with an exactitude of 0.5 mm., the largest diameter and the smallest perpendicular diameter were measured, and their mean noted as the diameter of the nucleus. The nuclear volume was calculated to $1/6\ \pi\ d^3$. — To avoid the »personal errors», mentioned by E. Freerksen, all the measurements were performed by one person (A. K.)

A binocular, $\times$ 10 and objective 40, was used for determining the mitotic ratio. This determination was done only in the outer half of the fascicular zone, but along the whole cut surface. The number of inspected visual fields was noted and, with the knowledge of the size of the visual field, the size of the inspected surface was calculated.

The t-test and the rank-sum test were used for statistical assessment of the material, the former for comparison of the nuclear volume and the latter for evaluation of the mitotic ratio. The rank-sum test was chosen because the mitotic distribution was highly asymmetric. The probability of the deviation (P) of the various values is given as highly significant, significant, and almost or possibly significant.

RESULTS

This investigation consists of two series: Test series I comprises 39 guinea-pigs, 5 of which were used for control, and Test series II includes 20 animals, 5 of which were used for control. The first series of tests was performed during the latter part of May to mid August, and the second was started early in November.

Nuclear Volume. — Table I shows that the mean volume of the nuclei in the fascicular zone was higher throughout in Test series I, examined during spring and summer, as compared with that of the animals in Test series II, examined in the latter part of the autumn. The mean figure for the nuclear volume in the controls of the two series was $149.3 \pm 4.7\ \mu^3$ and $132.4 \pm 4.4\ \mu^3$ and the difference was statistically significant.

In Test series I and II, the mean values for nuclear volume in the animals subjected to histamine treatment did not diverge statistically from the controls in the two series. The only statistically tenable

TABLE 1

TEST SERIES I: ANIMALS DYING IN ACUTE SHOCK AFTER THE 1ST INJECTION OF HISTAMINE IN BEESWAX, AND SUCH DECAPITATED 6 HOURS AFTER HISTAMINE TREATMENT FOR 12 OR 16 DAYS
TEST SERIES II: ANIMALS DECAPITATED 6 HOURS AFTER THE 1ST OR 3RD INJECTION OF HISTAMINE IN BEESWAX

n = number of animals, $\bar{x} \pm s\,\bar{x}$ = mean value for nuclear volume ± standard deviation (μ^3), $\bar{m}$ = mean value for mitotic ratio, $\bar{a}$ = mean value for slices studied (mm^2))

		n	$\bar{x} \pm s\,\bar{x}$	$\bar{m}$	$\bar{a}$
Test series I	Controls	5	149.3±4.7	2.4	8.1
	Died in acute shock	9	139.2±5.7	2.2	6.0
	Decapitated after 12 injections	18	151.5±2.9	3.4	7.8
	Decapitated after 16 injections	7	155.3±4.2	4.2	5.0
Test series II	Controls	5	132.4±4.4	2.4	6.9
	Decapitated after 1 injection	10	135.2±3.8	1.9	7.1
	Decapitated after 3 injection	5	133.1±4.0	9.8	5.6

difference established was the one observed in the animals dying from acute shock in connection with the first histamine injection and those decapitated after the 12th or 16th injection; in both cases the difference was significant. It should be noted here, however, that the animals succumbing to acute shock, died 20 minutes to one hour after the histamine injection.

Figs. 1 and 2 show the distribution graphs for the nuclei in the different size groups in Test series I and II. The abscissa indicates the nuclear diameter (μ) and the corresponding nuclear volume (μ^3). The ordinate shows the mean frequency ($\bar{f}$) for the nuclear classes in the various histamine tests.

It is seen that there is rather good agreement in the shape of the graphs, the maximum frequency being 2—3 size classes. In Test series I, the graph is distinctly shifted to the left in the case of the animals dying from acute shock after the first injection, and a broadening of the maximum frequency in the case of the animals given 16 histamine injections. The graphs for Test series II are all shifted to the left, as compared with those for Test series I. The graphs for the controls in the former series show, in addition, a more pronounced maximum for frequency by its higher apical shape and its thinning down base. As already mentioned, in the controls of the two test series, there was a statistically significant difference as to nuclear volume.

In a work published by W. Boguth *et al.* (5), to which we here refer, the difference in shape, height, shift to the left or right in the frequency graphs shows the influence of hypophysectomy and different kinds of stress on the nuclear volume in the fascicular zone. As our nuclear measurements gave no evidence in support of statistically tenable differences in the size of nuclei in the histamine tests, such as carried out in the present work, we must abstain from trying to analyse the comparatively small variations appearing in our graphs. In this connection, reference may be made also to the report of R. Dallwig (9). In his tests on rats, the stress consisted in 10 days' thirst, but no changes in the nuclear size in the fascicular zone were evidenced.

Mitotic Ratio. — At determination of the mitotic rate in the outer half of the fascicular zone, where the size of the nuclei was also measured, it was observed that comparatively few mitoses occurred in the controls of the two test series (Tab. 1); the rate was of the

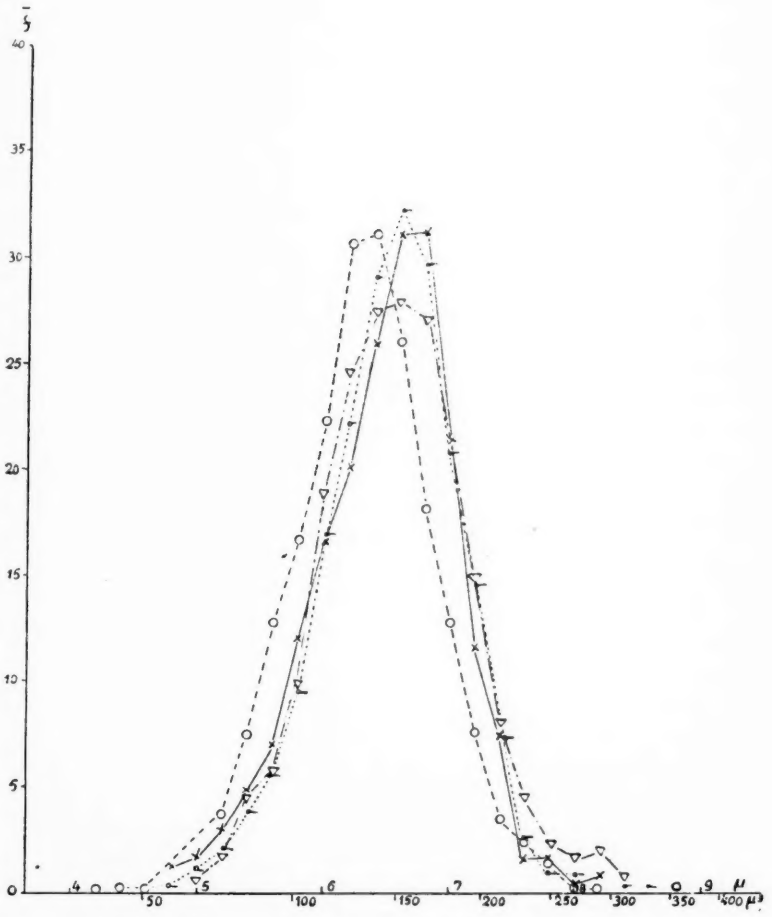


Fig. 1. — Test series I. Graph showing the distribution of nuclei in the different size groups. The abscissa show the diameter (μ) and the volume (μ^3) of the nuclei while the ordinate indicates the mean frequency ($\bar{f}$) of the nuclear classes.

×——× = controls,
○-----○ = died in acut shock,
♂·····♂ = decapitated after 12 injections,
▽—·—▽ = decapitated after 16 injections.

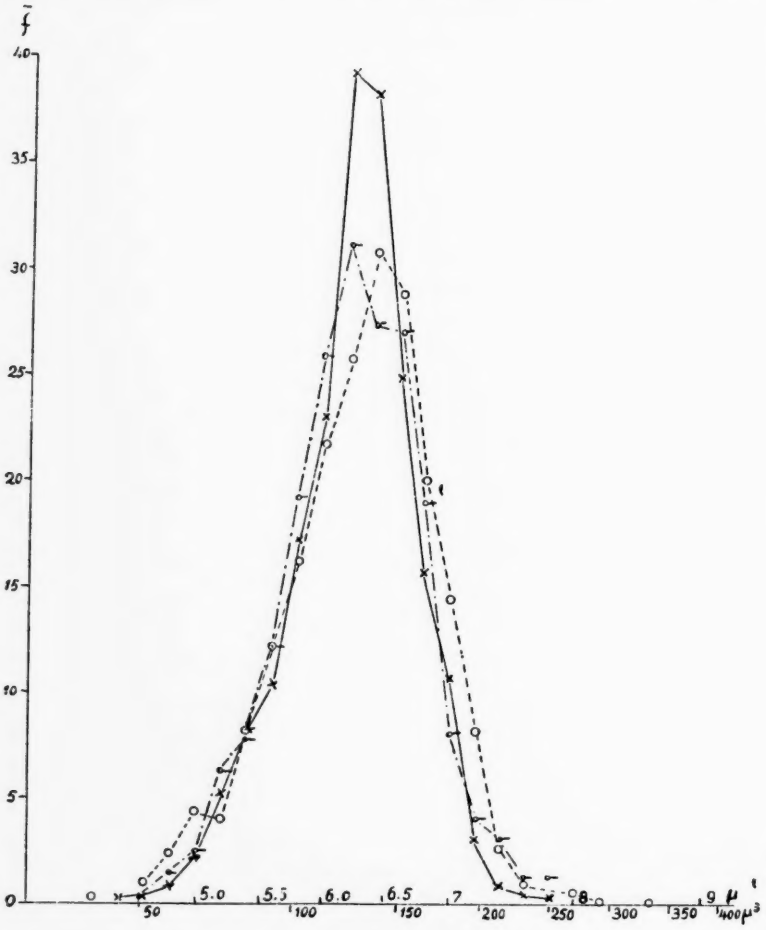


Fig. 2. — Test series II. Graph drawn on the same principles as Fig. 1.

×——× = controls,
○-----○ = decapitated after one injection,
♂— - —♂ = decapitated after three injections.

same size in the two series. Nor did any appreciable changes in the ratio occur after histamine treatment.

Statistically tenable differences were recorded by means of the rank-sum test, that is true, on comparison with the controls for animals dying from acute shock, those given three or 16 injections, but as the mitotic ratio for all the animals was very low, no signif-

icance should be attributed to these variations in this connection, especially as, for instance R. Dallwig's (9) mean mitotic ratio in the fascicular zone of rat was 5 with a deviation coefficient of $\pm$ 6, and after thirst 6 $\pm$ 7. H. T. Blumenthal (4) mentions a mitotic ratio of 4.1, on an average, per section of guinea-pig adrenal gland.

COMMENTS

In their tests on guinea-pigs, W. Boguth *et al.* (5) showed that different kinds of stress, such as hunger, penicillin and diphtheria toxin bring about an enlargement of the nuclei in the fascicular zone of the adrenal gland. This morphokinetic adaptation, released in accordance with Selye's General-Adaptation-Theory, did not appear in the experiments if the test animals were hypophysectomied before being exposed to stress, provoking an alarm reaction. R. Bachmann (1) likewise says, quoting C. M. Jackson (16), that the size of the nuclei in the outer fascicular zone of adult rats decreased by 19 per cent during a period of acute hunger of several days' duration, and that this reduction is further increased in periods of chronic hunger. Simultaneously, the mitotic ratio decreases. After saline stress on rats lasting four weeks, D. Eichner (11) observed an increase of 27 per cent in the nuclear volume in the outer fascicular zone. On the other hand, R. Dallwig (9), subjecting rats to thirst over 10 days, observed changes, neither in size of nucleus nor in mitotic ratio in the fascicular zone. As she noted alterations in both nuclear size and mitotic ratio in the glomerular zone at the same time, she concluded that the effect of thirst and administration of saline was relatively specific in this zone, whereas a similar action on the fascicular zone was improbable. Hence she considered that, for instance, D. Eichner's just mentioned observations of changes in the fascicular zone were caused by an unspecific stress action — in agreement with what has been previously said about the interpretation of the results obtained by W. Boguth *et al.* (5) — whereas R. Dallwig, as already mentioned, assumed that the changes seen in the glomerular zone might be due to increased secretion of Adiuretin in connection with thirst; her deduction was based on the studies of M. Kimura (17), Ch.Lloyd and J. Lobotsky (20), and R. Ortmann (26).

That the various cortical zones of the adrenal gland react differently to irritation agrees well with the relative independence attributed to them. But, again, an absolute independence is contradicted by, for instance, regeneration findings (16) and the progressive and regressive transformations (5) in the zones of the gland.

In spite of different kinds of stress thus causing a morphokinetic adaptation reaction, no definite results were established in this respect, for instance, changes in the nuclear volume, or influence on the mitotic ratio after histamine injections under the conditions prevailing. This is somewhat surprising as histamine is considered a particularly active stress agent which has been used for studies of experimental shock, and also an endogenous substance which is associated with different kinds of shock and anaphylactic behaviour. The absence of a stress reaction after histamine treatment of the animals might be due to its high toxicity — the lethal dose for guinea-pigs is as little as 0.3—0.4 mg/kg of body weight when injected intravenously (10) — which may be thought to inhibit the activity of the hypophysis and thus the production of ACTH. This assumption may be supported by the tendency observed that the animals succumbing during acute shock after the first histamine injection revealed a statistically significant decrease in nuclear volume on comparison with those given histamine injections over a period of 12 or 16 days. It might be thought, also, that the histamine might exercise its toxic action on the adrenal gland directly — according to the theory regarding the direct action of histamine on the cells — which, by this means, might become refractory to a stress effect mediated by the hypophysis. On the other hand, the results of the investigation, with regard to the size of the nucleus, seem to show that there is a tendency suggesting that the adrenal gland adjusts itself to increased responsiveness, in accordance with Selye's General Adaptation Theory, although this is not mathematically provable. The results obtained in the present investigation agree in some measure with those obtained in studies on the effect of histamine on the thyroid gland, where the activity of the gland is not affected (24, 33). This differs from conditions during anaphylactic shock (8), and using stressors, such as alloxan and formaldehyde (28, 29).

SUMMARY

The adrenal glands of 59 male guinea-pigs were examined. Of these, 39, including controls, were treated in spring and summer (Test series I) and 20, likewise including controls, in autumn (Test series II). In both series the animals were given one daily intramuscular injection of histamine embedded in beeswax and paraffin oil in doses equivalent to about 2 mg of histamine base. In the first series, the animals were given histamine over periods of 12 or 16 days, and in the second series for 2 or 3 days. The animals dying from acute shock after the first injection belong to Tests series I.

Differences neither in size of nucleus nor mitotic ratio in the fascicular zone of the adrenal gland were observed on comparison of the animals treated with histamine and the controls. However, there may perhaps have been some tendency to changes in the nuclear volume, to judge from the fact that the animals dying from acute histamine shock after the first injection had a significantly smaller nucleus than those treated with histamine over a period of 12 or 16 days. — The guinea-pigs treated in summer and spring had larger nuclei in the fascicular zone than those treated in autumn.

REFERENCES

1. Bachmann, R.: Die Nebenniere. Hdb. d. mikr. Anat. d. menschen 1954:*6*, as referred to by Dallwig.
2. Bizzozero, G.: Anat. Anz. 1888:*3*:781.
3. Bizzozero, G.: Arch. mikr. Anat. 1893:*42*:82.
4. Blumenthal, H. T.: Endocrinology 1940:*27*:477.
5. Boguth, W., Langendorff, H., and Tonutti, E.: Med. Welt 1951:*13*:408.
6. Bullough, W. S.: Phil. Trans. Royal Soc. London 1946:*231*:453, as referred to by Dallwig.
7. Caspersson, T., and Holmgren, Hj.: Anat. Anz. 1934:*79*:53.
8. Costa, A., Volterrani, O., Marchis, E., Benedetto, V., and Scorta, A.: Boll. Soc. ital. biol. sper. 1952:*28*:557, as referred to by Excerpta Medica Sectio 3, 1953:*7*:325.
9. Dallwig, R.: Ztschr. f. mikr.-anat. Forsch. 1954:*61*:138.
10. Doerr, R.: Die Immunitätsforschung. Die Anaphylaxie 11. Immunitätsreaktion und endogene Vergiftung. Springer-Verlag, Wien 1951.
11. Eichner, D.: Ztschr. f. Zellforsch. 1953:*38*:488.

12. FREERKSEN, E.: Ztschr. f. Zellforsch. 1933:*18:*362.
13. GÜNTHER, H.: Endocrinologie 1941/42:*24:*290.
14. HARTMAN, F., and BROWNELL, K. A.: The Adrenal Gland. Lea & Febiger, Philadelphia 1949.
15. HAY, L. J., VARCO, R. L., CODE, CH. F., and WANGENSTEEN, O. H.: Surg. Gynec. Obst. 1942:*75:*170.
16. JACKSON, C. M.: Am. J. Anat. 1915:*18:*75.
17. JONES, J. CH., and JONES, M.: J. Endocrin. 1954:*10:*245.
18. KIMURA, M.: Japan J. Physiol. 1954:*4:*24, as referred to by DALLWIG.
19. KLEIN, H.: Virchows Arch. 1951:*320:*93.
20. KLEIN, H., and GEISEL, H.: Klin. Wschr. 1946/47:*24:*662.
21. LLOYD, CH., and LOBOTSKY, J.: J. Clin. Endocrin. 1950:*10:*318.
22. LÖFGREN, L.: Ann. Med. exp. Biol. fenn. 1954:*32:*Suppl. 6.
23. LÖFGREN, L.: Acta Anat. 1956:*26:*62.
24. LÖFGREN, L., and ISOTALO, A.: Ann. Med. exp. Biol. fenn. 1954:*32:*56.
25. LÖFGREN, L., and RYTÖMAA, T.: Acta Anat. 1957:*31:*376.
26. NICOLAY, P.: Boll. Soc. ital. biol. sper. 1939:*14*, as referred to by TEIR.
27. ORTMANN, R.: Verhandl. Anat. Ges. 1954:*52:*95, as referred to by DALLWIG.
28. PASCHKIS, K. E., CANTAROW, E., EBERHARD, T., and BOYLE, D.: Proc. Soc. Exp. Biol. & Med. 1950:*73:*116.
29. SELYE, H.: The Physiology and Pathology of Exposure to Stress. — Acta Inc. Medical Publishers, Montreal 1950.
30. SELYE, H.: Annual Report on Stress. — Acta Inc. Medical Publishers, Montreal 1951.
31. SELYE, H.: Endocrinology 1951:*49:*197.
32. TEIR, H.: Acta path. microbiol. Scandinav. 1944:Suppl.51.
33. VOLTERRANI, O., and MARCHIS, E.: Folia Endocrinol. 1952:*5:*189.

FROM THE DEPARTMENT OF PATHOLOGY, SECTION II, UNIVERSITY OF HELSINKI

EFFECT OF ALCOHOLS ON EOSINOPHILE CELLS IN THE GLANDULAR STOMACH OF THE RAT[1]

by

T. MIETTINEN and K. KOUVALAINEN

(Received for publication July 31, 1958)

Fluctuations in the eosinophilic cells of the blood and also of the tissues during Selye's stress syndrome are generally known, but the physiological or pathological significance of these changes is still obscure. Many of the changes in the behaviour of eosinophils towards noxa have generally been explained as the result of general stress, but on the other hand the eosinophils are considered to have a directly protective action against noxa (5, 8). In an earlier study we observered that benzpyrene dissolved in olive oil and allowed to act locally for a short time caused an increase in the eosinophil count in the rat glandular stomach (2).

According to Richardson's rule, the effect and toxicity of different alcohols increases with increasing molecular size. The mucosa of the dog's stomach has been found to be more greatly damaged by propyl, butyl and amyl alcohols than by ethyl alcohol (1). The reaction produced by an intracutaneous injection of alcohol has been observed to follow the homologous series in the different alcohols (4). Ethyl alcohol has a certain effect on blood eosinophils which is antagonistic to cortisone (3).

Certain factors which influence the secretion of digestive juices have been found to cause eosinophilia in the digestive tract of

[1] Sponsored by the Sigrid Jusélius Fondation.

the rat (8). Since ethyl alcohol is known to increase the secretion of gastric juice, its possible effect on eosinophils in the stomach appeared also for this reason to be of interest.

MATERIAL AND METHODS

The experimental animals were male Sprague-Dawley rats 3—4 months old, who had been kept all the time under the same conditions and on the same diet. For 2 days before experiment the rats fasted and were given water only. Seven- to ten rats were used in each experiment, except in studying the effect of amyl alcohol during 1 hour, for which 19 rats were used, since we wished to confirm the significance of the mild eosinophilia observed in preliminary experiments.

The substances under test as well as the corresponding amount of physiological saline solution for the controls were cannulized into the stomach by means of a rubber cannula. The time of exposure was 1 and 4 hours, after which the rats were killed with ether and a biopsy specimen was taken from the stomach.

The preparation of the microscopic specimens and the counting of eosinophils from the lamina propria of the glandular stomach was done in the manner described in our earlier paper (2). The number of eosinophils are expressed per 0.05 sq.mm, although the counting was performed over an area of twice this size. Lesions in the mucosa produced by the alcohols were observed macroscopically and microscopically.

The substances and doses used were as follows:

Methyl alcohol	2	ml.	30	per cent
Ethyl alcohol	2	»	30	» »
Propyl alcohol	0.5	» +	1.5	ml. H_2O
Butyl alcohol	0.5	» +	1.5	» »
Amyl alcohol	0.5	» +	1.5	» »

RESULTS

The control groups in all the experimental series showed higher values at 4 hours than at 1 hour, although the difference was not significant.

Compared with the controls (1 hr.: 59.6 ± 15.5 and 4 hrs.: 76.1 ± 30 eos./0.05 sq.mm.) no difference was seen in the number of eosinophils in rats treated with methyl alcohol (1 hr.: 69.5 ± 21.3 and 4 hrs. 68.8 ± 12.9 eos./0.05 sq.mm), ethyl alcohol (69.5 ± 32.6 and 71.5 ± 7.2), propyl alcohol (57.5 ± 32.5 and 74.2 ± 16.3) and butyl alcohol (61.0 ± 24.4 and 104.6 ± 36.0).

The results obtained with amyl alcohol are shown in fig. 1. This substance was found to cause a moderate increase in the eosinophils in the glandular stomach within 1 hour ($P < 0.05$). After exposure for 4 hours the increase was not significant (96.0 ± 22.6). The stomach of the rats given amyl alcohol was markedly more relaxed and dilated than that of the other rats, which

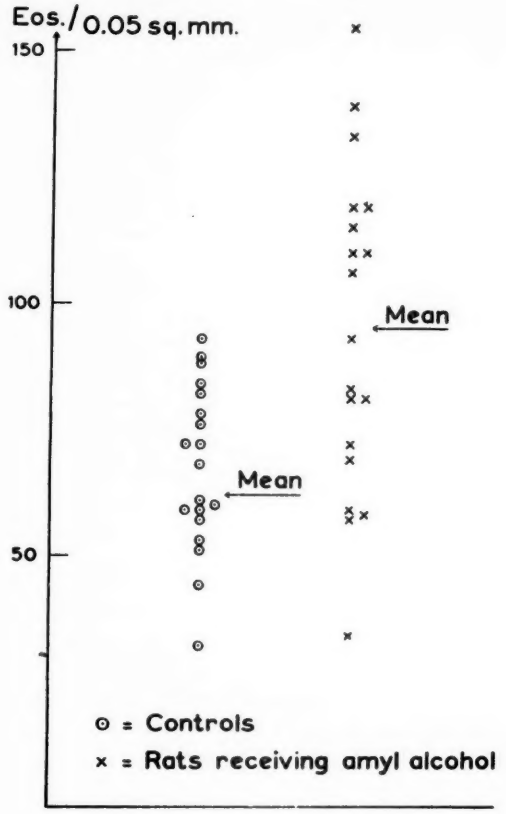


Fig. 1. — Effect of amyl alcohol on the number of eosinophils in the glandular stomach of the rat after exposure for 1 hour.

seemingly reduced the number of eosinophils per unit area in these rats because of the counting method employed. Thus the actual change produced by amyl alcohol is greater than shown by our figure.

Small granulocyte accumulations and a few small ulcerations were encountered to some extent in all the groups but no quantitative differences were observed between the various alcohols in the homologous series or between 1 and 4 hours exposures.

DISCUSSION

Although no graduated correlation was observed in these experiments between the molecular size of the alcohols applied locally to the stomach and the eosinophil count in the lamina propria, attention is drawn to the increase in the eosinophil count produced by amyl alcohol in 1 hour. Amyl alcohol is the most largemolecular and toxic of the substances in the homologous alcohol series used. It is possible that larger doses would have produced changes in also the other experimental groups. However, the dosages already were relatively large. The letal doses for these alcohols are as follows (6):

Methyl alcohol LD	50	appr.	12.9	gm./kg.	orally	
Ethyl alcohol	50	»	13.7	»	»	»
Propyl alcohol	50	»	1.9	»	»	»
Butyl alcohol	50	»	4.4	»	»	»
Amyl alcohol LD		»	0.5	»	»	intraperitoneally.

On the basis of the results obtained in the present study it does not appear improbable that eosinophils have an antitoxic action (5, 8) but in this case the explanation of general stress and accordingly of increased adrenocortical hormone secretion (7) seems more plausible, since the rats which were given amyl alcohol were clearly under a definitely greater stress during the experiment, exhibiting greater difficulty of breathing and somnolence than the other rats.

Macroscopic and microscopic lesions in the mucosa of the glandular stomach were unexpectedly few. For example, the gastric mucosa of the dog has been found to be injured more greatly by largemolecular alcohols than by ethyl alcohol (1). In these experiments we were unable to make a corresponding finding in rats. In the guinea pig ethyl alcohol has produced various gastric lesions

only in concentrations of 60 per cent and over, but then already in onehalf hour (9). We used a 30 per cent concentration of ethyl alcohol.

SUMMARY

This report covers a study of the effect of perorally given methyl, ethyl, propyl, butyl and amyl alcohols on the glandular stomach of the rat by determining, specifically the number of eosinophile cells in the lamina propria. Amyl alcohol was found to produce an increase in the number of eosinophils within 1 hour. Unexpectedly few lesions were found in the gastric mucosa and they revealed no difference in number or type in the rats given different kinds of alcohol and after the different periods of effect.

REFERENCES

1. Kast, L.: Arch. f. Verdauungskr. 1906:*12:*487, cited by Kochmann, M.: Handbuch der Experimentellen Pharmakologie.
2. Kouvalainen, K., and Miettinen, T.: Ann. Med. Exper. Fenn. 1956:*34:* 375.
3. Laves, W.: Arch. Exper. Path. u. Pharmakol. 1954:*222:*482.
4. Renkonen, O., and Teir, H.: Ann. Med. Exper. Fenn. 1957:*35:*67.
5. Schlecht, H.: Deutsche Med. Wchnschr. 1944:*70:*189.
6. Spector, W. S.: Handbook of Toxicology. Vol. I, Philadelphia 1956.
7. Teir, H., and Wegelius, O.: Acta Endocrinol. 1958:*28:*197.
8. Teir, H., Wegelius, O., Sundell, B., Päivärinne, I., and Kuusi,T.: Acta Med. Scand. 1955:*152:*275.
9. Williams, A. W.: Brit. Med. J. 1956:*252:*4961.

FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

BACTERIA FOUND IN URINE

by

ILMARI PALVA

(Received for publication July 1, 1958)

Urinary tract infections are still fairly common and show a great tendency to linger on (2) in spite of the improved therapeutical facilities available now, above all the new sulfa drugs and antibiotics. In the Finnish literature, reports on bacteria isolated from urine have been published by H. Streng in 1936 (6), Tuovinen in 1949 (7) and Rantasalo in 1953 (3).

The present paper is a report on the 7,271 strains of microorganisms isolated from 20,903 urine samples at the Department of Serology and bacteriology of the University of Helsinki in the five-year period 1951—55. The present situation is compared with earlier analyses.

Table 1 reflects the total material. All together 2,104 strains of Gram-positive cocci was encountered, *i.e.* nearly a third of the total bacterial flora. There were 819 Staphylococcus epidermidis and 536 Staphylococcus aureus strains. The Staphylococcus group thus totalled 1,355 strains, *i.e.*, 18.6 per cent of all the bacteria. The Streptococci in the material accounted for only about a half of this figure. The following strains were found: Streptococcus faecalis 582, Streptococcus *a*-haemolyticus 103, *β*-haemolyticus 10 and others 54 (10.3 per cent).

Gram-negative rods represented a good two-thirds (69.3 per cent) of the total material. Half of this quantity, 2,443 strains, consisted of Escherichia coli. Next in order of frequency came the Klebsiella-Aerogenes group with 784 strains (10.8 per cent). Thirh

TABLE 1

DISTRIBUTION OF 7,271 MICRO-ORGANISM STRAINS ISOLATED FROM URINE

Micro-organism	Strains				Per Centages of Strains in Mixed Cultures
	Pure Cultures	Mixed Cultures	Total		
			Number	Per Cent	
Staphylococcus epidermidis ..			819		
Staphylococcus aureus			536		
Staphylococci together	980	375	1355	18.6	28
Streptococcus faecalis			582		
Streptococcus *a*-haemolyticus			103		
Streptococcus *β*-haemolyticus			10		
Other Streptococci			54		
Streptococci together	448	301	749	10.3	40
Gram + cocci together	1428	676	2104	28.9	32
Escherichia coli	2217	226	2443	33.6	9
Escherichia freundii	3	—	3	0.04	—
Klebsiella-Aerogenes	681	103	784	10.8	13
Salmonella paratyphi B			4		
Salmonella typhi murium....			11		
Salmonella, not subtyped			4		
Salmonellae together	17	2	19	0.26	11
Proteus mirabilis			239		
Proteus vulgaris			103		
Proteus rettgeri			38		
Proteus morganii			29		
Proteus, not subtyped			154		
Proteus together	448	115	563	7.8	20
Other coliforms	272	120	392	5.4	31
Pseudomonas	233	123	356	4.9	35
Faecalis alkaligenes	177	50	227	3.1	22
Gram — rods, not finally classified	173	75	248	3.4	30
Gram — rods together	4201	834	5035	69.3	17
Gram +, spore-forming.....	3	1	4	0.05	25
Diphteroid	30	32	62	0.85	52
Clostridium	—	1	1	0.01	100
Gram + rods together	33	34	67	0.91	51
Candida	44	21	65	0.89	32
In all	5706	1565	7271	100	20

TABLE

BACTERIAL FLORA IN THE URINE

Author and Period Covered by the Material	Number of Strains	Staphylococci Per Cent	Streptococci Per Cent
H. Streng 1931	436	21	18*
Tuovinen 1936—38	407	23	4**
1946—48	271	25	9**
Rantasalo 1951	841	12	19
Palva 1951—55	7271	19	10

* »saprophytes of the urethra»
** »other bacteria»

(7.8 per cent) came the 563 strains of the genus Proteus of which 154 were not finally subtyped. Of the subtyped strains 59 per cent were P. mirabilis, 25 per cent P. vulgaris, 9 per cent P. rettgeri and 7 per cent P. morgani. The group »other coliforms» included B. paracoli, Ballerup-Bethesda, Alcalescens dispar, Providence, Serratia, Arizona, Cloaca cloacae and Erwinia. They represented a total of 392 strais in the material, *i.e.*, 5.4 per cent. The Pseudomonas strains found totalled 356 (4.9 per cent) and Faecalis alkaligenes 227 (3.1 per cent). Of Gram-negative rods, 248 strains (3.4 per cent) were not finally classified. Salmonella (19 strains) and Escherichia freundii (3 strains) were found sporadically.

Other bacteria were fairly rare. Diphteroids were found 62 times (0.85 per cent). The bacterial flora included 4 strains of Gram-positive, spore-forming rods and a single Clostridium. The results of Mycobacterium tuberculosis cultures were not taken into consideration.

Candida strains were present in 65 samples.

In mixed infections the streptococci seemed to be very common, but the E. coli relatively few. Mixed infections with two strains consisted of nearly all the possible combinations. Strains isolated from mixed infections accounted for 1,565 (20 per cent) of the total material.

Table 2 contains the data reported by H. Streng, 1936, Tuovinen, 1949 and Rantasalo, 1953 and those of the present author. There were no significant differences between them. The group »Coli» of the former two workers corresponds well to the group of Gram-negative rods of the latter authors. Noteworthly, the

2

IN FINLAND IN 1931—1955

Gram + Cocci Per Cent	E. Coli Per Cent	Proteus Per Cent	Other Gram-rods Per Cent	Total of Gram-rods Per Cent
39	55	6	—	61
27	65	—	8	73
34	47	—	19	66
31	21	7	38	66
29	34	8	27	69

Proteus frequency has remained practically the same: 6 per cent in Streng's, 7 per cent in Rantasalo's and 8 per cent in the present material. The proportion of Staphylococci seems also to have remained fairly constant, around 20 per cent. However, Rantasalo isolated in 1951 at the State Serum Institute clearly fewer Staphylococci than but twice as many Streptococci (19 per cent) as we did. In materials published elsewhere in the last few years (1, 4, 5) the ratio of the totals of Gram-negative rods and Gram-positive cocci is on the whole the same as in Finland but there are still great differences between different laboratories in the more detailed classification of these groups.

SUMMARY

Judging by the distribution of the 7,271 micro-organisms isolated from urinary tract infections the most common cause of these infections in Finland today is E. coli which accounted for a third of these micro-organisms. Staphylococci represented nearly 20 and Streptococci 10 per cent. Klebsiella bacteria accounted for c. 10, Proteus for c. 8 and other coliforms and Pseudomonas for c. 5 per cent each. For Faecalis alkaligenes the percentage was c. 3. There were incidental findings (less than 1 per cent) of Diphteroids, Salmonellae, Gram-positive spore-formers, Clostridia and Candidae. Nearly all the possible combinations were met with in mixed infections.

No essential change in the bacterial flora of urinary tract infections has occurred in Finland during the last twenty-five years.

REFERENCES

1. Böhne, C., and Schulze, W.: Die Medizinische 1953: p. 747.
2. Järvinen, K. A. J.: Suomen Lääkärilehti 1958:*13:*493.
3. Rantasalo, I.: Suomen Lääkärilehti 1953:*8:*954.
4. Rhoads, P. S., Billings, C. S., and O' Conor, V. J.: J.A.M.A. 1952: *148:*165.
5. Secrétan, M.: Schweiz. Med. Wschr. 1952:*82:*345.
6. Streng, H. O.: Duodecim Ser. A Tom XIX, 1936.
7. Tuovinen, P. I. Ann. med. exp. & biol. Fenn. 1949:*27:*102.

FROM THE DEPARTMENT OF MEDICAL CHEMISTRY, UNIVERSITY OF HELSINKI

1952:

EFFECT OF ACETONE ON THE METABOLISM OF METHANOL IN RAT LIVER HOMOGENATES[1]

by

MARTTI KOIVUSALO

(Received for publication August 27, 1958)

Asser (2), working with dogs and rabbits, found in 1914 that acetone, ethanol and amyl alcohol diminished the increased excretion of formic acid in the urine resulting from the ingestion of methanol. He considered this effect to be due to an increased oxidation of formic acid. The effect of ethanol on the metabolism of methanol has since been studied rather extensively but the possibly similar effect of acetone has not been much studied. In experiments with rabbits (7), the simultaneous administration of acetone retarded the elimination of methanol from the blood only very slightly when compared with the retarding effect of ethanol in similar experiments (6). The present paper deals with the effect of acetone on the utilization of methanol and on the accumulation of formaldehyde in rat liver homogenates incubated with methanol.

METHODS

The experimental animals were white rats of Wistar strain weighing 280—320 g. They were fed the usual stock diet of this Department.

After the killing of the animals, their livers were rapidly removed, cooled on ice, weighed and homogenized to a 10 per cent homogenate

[1] This investigation has been supported by a generous grant from the Foundation for the Research of Alcohol Problems.

in a cooled Bühler tissue homogenizer. The homogenising medium was ice-cold 0.1 M potassium phosphate buffer pH 7.4.

The acetone and methanol used were redistilled *pro analysi* products of E. Merck, Darmstadt

The incubations were carried out in a temperaturecontrolled water bath at 37° with constant shaking. In all the experiments the gas phase in the flasks was air. Samples were taken from the incubation flasks before and after the incubation. In some experiments a number of samples were taken also during the incubation. The samples of 1 ml were transferred to centrifuge tubes containing 5 ml of 10 per cent trichloroacetic acid for the precipitation of proteins. The clear supernatant obtained after centrifugation was used for the determinations.

The samples were analysed for their methanol and formaldehyde contents, using photometric methods (6) based on the oxidation of methanol with acid permanganate to formaldehyde and on the determination of the formaldehyde by the aid of the chromotropic acid reaction. The presence of acetone in the quantities used did not affect the determinations of methanol or formaldehyde.

RESULTS

The results of a typical series of experiments, where the reactions were followed during 120 minutes, are presented in figures 1 and 2. It is seen that acetone concentrations of 13—27 mg% in the incubation mixture had no effect on the utilization of methanol in rat liver homogenate. The presence of acetone increased slightly the accumulation of formaldehyde. Also no effect of acetone on the time course of these reactions was seen.

Although the concentration of acetone in the incubation mixture was increased up to 686 mg%, no effect was seen on the utilization of methanol (Table 1). The slight increase in the accumulation of formaldehyde when acetone was present was seen also in these experiments. When acetone alone was incubated with rat liver homogenate no appreciable accumulation of formaldehyde was observed.

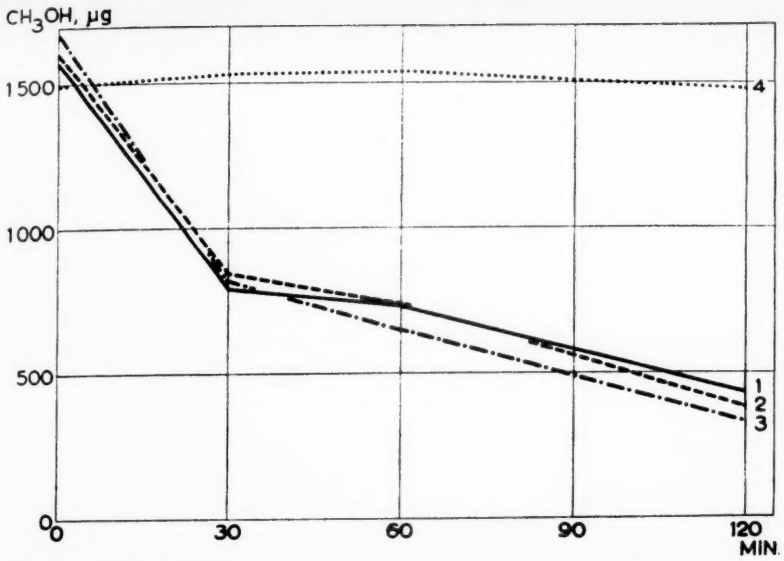


Fig. 1. — Effect of acetone on the utilization of methanol in rat liver homogenate. Ordinate: Concentration of methanol per initial volume. Abscissa: Incubation time. 10 ml of 10 per cent homogenate in 0.1 M potassium phosphate buffer pH 7.4. Total volume 12 ml. Incubated at 37°. Gas phase air

1600 µg of methanol
1600 µg of methanol (boiled homogenate).
1600 µg of methanol + 1600 µg of acetone
1600 µg of methanol + 3200 µg of acetone

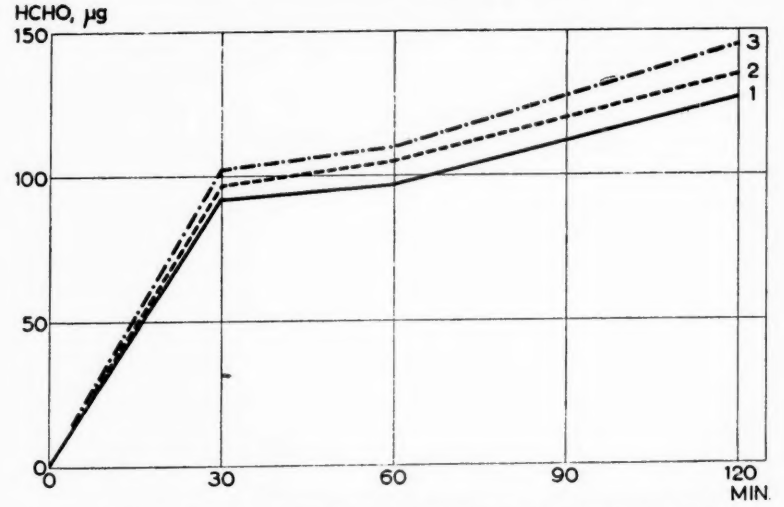


Fig. 2. — Effect of acetone on the accumulation of formaldehyde in rat liver homogenate incubated with methanol. Ordinate: Amount of formaldehyde accumulated. Abscissa: Incubation time. Same experiments as in fig. 1. No formaldehyde accumulated in the boiled homogenate incubated with 1600 µg of methanol.

DISCUSSION

In the earlier *in vivo* experiments (7) with rabbits the simultaneous administration of acetone retarded the elimination of methanol from the blood, although rather slightly and only when the ratio of acetone to methanol was 1 : 1 or more. In the present *in vitro* experiments, acetone had no retarding but, if any an increasing effect on the utilisation of methanol in the rat liver homogenates, although the ratio of acetone to methanol was increased as high as 60 : 1.

TABLE 1

EFFECT OF ACETONE ON THE UTILIZATION OF METHANOL AND ACCUMULATION OF FORMALDEHYDE IN RAT LIVER HOMOGENATES

5 ml of 10 per cent homogenate in 0.1 M potassium phosphate buffer pH 7.4. Total volume 7 ml. Incubated for 60 minutes at 37°. Gas phase air. Each value is the average of two incubation flasks. Different homogenates are used in experiments 1—6 and 7—12.

	Methanol Utilized μg	Formaldehyde Accumulated μg
1 800 μg methanol	365	59
2 800 » » + 3.2 mg acetone	380	66
3 800 » » + 6.4 » »	390	69
4 800 » » + 9.6 » »	385	66
5 3.2 mg acetone	—	1
6 6.4 » »	—	0
7 800 μg methanol	350	40
8 800 » » + 8.0 mg acetone	380	47
9 800 » » + 24 » »	362	47
10 800 » » + 48 » »	390	52
11 24 mg acetone	—	2
12 48 » »	—	2

Acetone is known to be utilized in the animal organism, although this takes place rather slowly (1, 3—5, 9—11, 13—15). The formation of formaldehyde during the metabolism of acetone should be possible, since acetone is known to take part in the so-called »one carbon group» reactions. Formate has been shown to be formed from the methyl carbons of acetone, which also may be used for all the reactions of the labile methyl group (13, 14). The intermediary reactions are not known with certainty. Formate is pre-

sumably formed from the cleavage of acetone to »formate» and »acetate». The latter may be used via acetyl-CoA for all the reactions of the »active acetate» (3, 4). Other known pathways for acetone in the animal organism are carboxylation to acetoacetate (10), which links it to the β-methyl β-hydroxyglutaryl-CoA cycle of Lynen *et al.* (8), and a direct formation of a triose-like compound (5). Propanediol phosphate has also been found to be a possible intermediate in the reactions of acetone (12).

When acetone alone was incubated with rat liver homogenates no formaldehyde or only negligible amounts could be recovered from the incubation mixture in the present experiments. This result does not, however, exclude the possible formation of formaldehyde during the metabolism of acetone. The formed small amounts of the very reactive formaldehyde may have been reacted further as soon as they were formed. The slightly increased formation of formaldehyde from methanol, when acetone was also present was apparently due to the likewise increased utilization of methanol and not to any noteworthy formation of formaldehyde from acetone.

According to Asser (2), acetone and ethanol had a similar decreasing effect on the increased excretion of formic acid in the urine of dogs after the administration of methanol. This effect of ethanol has later been confirmed by several authors. Simultaneous administration of ethanol retarded very clearly the elimination of methanol from the blood of rabbits, and ethanol was also shown to inhibit competitively the utilisation of methanol in rat and guinea pig liver homogenates.

The earlier *in vivo* experiments and also the present *in vitro* experiments did not show a similar effect of acetone on the metabolism of methanol as that attained with ethanol. Thus the parallelism presumable from the results of Asser between the effects of acetone and ethanol on the metabolism of methanol does not seem to be generally valid.

SUMMARY

The effect of acetone on the utilization of methanol and accumulation of formaldehyde was studied in rat liver homogenates incubated with methanol.

In contrast to the earlier *in vivo* experiments, no retardation but a slight increase in the utilization of methanol was found even though the ratio of acetone to methanol was increased to 60 : 1. A small increase in the accumulation of formaldehyde was also consistently found when acetone was present with methanol. When acetone alone was incubated with rat liver homogenates no formaldehyde or only a negligible amount was formed.

REFERENCES

1. Antognetti, L., and Scopinaro, D.: Arch. »E. Maragliano» Pat. Clin. 1946:*1:*2.
2. Asser, E.: Z. Exp. Pathol. Therap. 1914:*15:*322.
3. Borek, E., and Rittenberg, D.: J. Biol. Chem. 1949:*179:*843.
4. Brady, R. O., and Gurin, S.: J. Biol. Chem.: 1951:*189:*371.
5. Hobbs, D. C., Hill, R. J., and Koeppe, R. E.: Fed. Proc. 1957:*16:*196.
6. Koivusalo, M.: Acta physiol. scand. 1956:*39:* Suppl. 131.
7. Koivusalo, M., Laukkanen, R., and Savolainen, A.: Ann. med. exp. biol. Fenn. 1958:*36:*22.
8. Lynen, F., Henning, U., Bublitz, C., Sörbo, B., and Kröplin-Rueff, L.: Biochem. Z. 1958:*330:*269.
9. Plaut, G. W. E., and Lardy, H. A.: J. Biol. Chem. 1950:*186:*705.
10. Polonovski, M., Valdiquie, P., and Teychenne, J.: Bull. soc. chim. biol. 1951:*33:*475.
11. Price, T. C., and Rittenberg, D.: J. Biol. Chem. 1950:*185:*449.
12. Rudney, H.: J. Biol. Chem. 1954:*210:*361.
13. Sakami, W.: J. Biol. Chem. 1950:*187:*369.
14. Sakami, W., and Lafaye, J. M.: J. Biol. Chem. 1951:*193:*199.
15. Scopinaro, D., Ghiani, P., and DeCecco, A.: Il Policlinico 1947:*54:*70.

FROM THE DEPARTMENT OF MEDICAL CHEMISTRY, UNIVERSITY OF HELSINKI

FORMATION OF FORMALDEHYDE FROM METHANOL IN RAT LIVER HOMOGENATES

EFFECT OF COFACTORS[1]

by

MARTTI KOIVUSALO

(Received for publication August 26, 1958)

Methanol can be metabolized in rat liver homogenates forming formaldehyde as an intermediate, which in part accumulates in the incubation mixture. No accumulation of formaldehyde could be shown during the metabolism of methanol *in vivo*, the formaldehyde being presumably metabolized further as rapidly as it is formed (4). In the former experiments in which various metabolic cofactors were added to fresh undialysed rat liver homogenates, no consistent effects on the formation of formaldehyde from methanol could be obtained (4). In the present paper results from experiments with aged, dialysed and Dowex—1 resin treated homogenates are presented. The effect of diphosphopyridine nucleotide, adenosine triphosphate and Mg^{++} and Mn^{++} ions is especially studied. Also semicarbazide and xanthine have been added in some experiments.

METHODS

The experimental animals were white female rats of Wistar strain weighing 260—280 g. They were fed the usual stock diet of this Department.

The animals were killed with a blow on the head, their livers were rapidly removed, cooled on ice and homogenized to a 10 per

[1] This investigation has been supported by a generous grant from the Foundation for the Research of Alcohol Problems.

cent homogenate in a cooled Bühler tissue homogenizer. Ice cold 0.1 M potassium phosphate buffer, pH 7.4 was used as homogenizing medium.

»DPN reinst» and ATP were products of Boehringer. All other reagents were pro analysi products of E. Merck, Darmstadt.

The homogenates were divided in two parts. The one part was dialysed for 24 hours at +4° against 0.1 M potassium phosphate buffer. The other part was also held at +4° for 24 hours.

The Dowex-treated enzyme was prepared as follows: 50 ml of the 10 per cent homogenate were stirred at +4° for 30 minutes with 5 g of Dowex—1 strongly basic anion exchange resin (X—10, chloride form, 200—400 mesh). After this the resin was removed by centrifugation.

The incubations were carried out in a temperature controlled water-bath with constant shaking at 37°. Air was used as gas phase in all experiments. Samples were taken from the incubation flasks before and after the incubation. The samples of 1 ml were transferred to centrifuge tubes containing 5 ml of 10 per cent trichloroacetic acid for the precipitation of proteins. The clear supernatant obtained after centrifugation was used for the determinations.

The samples were analysed for their formaldehyde content using a photometric method based on the chromotropic acid reaction (4).

All the values in the tables are averages of duplicate determinations from three similar incubation flasks.

RESULTS

The results of typical series of experiments are presented in Tables 1—4.

From the results recorded in Table 1 it is seen that in undialysed fresh rat livér homogenate addition of diphosphopyridine nucleotide had no appreciable effect on the accumulation of formaldehyde during incubation with methanol. Addition of adenosine triphosphate slightly increased the accumulation of formaldehyde. When semicarbazide was added in the incubation mixture more of the formed formaldehyde was trapped and the accumulation increased about 50 per cent.

When the liver homogenate was dialysed against phosphate

TABLE 1

EFFECT OF SEMICARBAZIDE, DPN AND ATP ON THE ACCUMULATION OF FORMALDEHYDE IN UNDIALYSED RAT LIVER HOMOGENATES INCUBATED WITH METHANOL

Additions	Formaldehyde accumulated μg
None	67
DPN	70
ATP	78
Semicarbazide	98

5 ml of 10 per cent homogenate in 0.1 M potassium phosphate buffer pH 7.4. Substrate 800 μg of methanol. Additions: 2.5 μM DPN; 5 μM ATP; 100 μM semicarbazide. Total volume 7 ml. Incubated for 60 minutes at 37°. Gas phase air

buffer for 24 hours less formaldehyde accumulated than in a fresh homogenate. The addition of diphosphopyridine nucleotide increased the accumulation of formaldehyde in the dialysed homogenate, but the addition of adenosine triphosphate caused a still greater increase. When both adenosine triphosphate and diphosphopyridine nucleotide were added, the effect was slighter than with adenosine triphosphate alone. The same effects were also seen in aged, undialysed homogenate but less clearly as in the dialysed homogenate. When

TABLE 2

EFFECT OF ATP AND DPN ON THE ACCUMULATION OF FORMALDEHYDE IN VARIOUSLY TREATED RAT LIVER HOMOGENATES INCUBATED WITH METHANOL

Additions	No Dowex Treatment		Treated with Dowex	
	Nondialysed	Dialysed	Nondialysed	Dialysed
None	29	12	21	8
ATP	93	107	—	116
DPN	44	27	—	27
ATP + DPN	90	88	—	97
None	37	24	—	—
ATP	68	107	—	—
DPN	52	66	—	—
ATP + DPN	98	96	—	—

Experimental conditions as in Table 1. Additions: 8 μM ATP; 2 μM DPN. The values given represent the amount of formaldehyde accumulated in μg.

TABLE 3

EFFECT OF MG++ AND MN++ IONS ON THE ACCUMULATION OF FORMALDEHYDE IN DIALYSED RAT LIVER HOMOGENATES INCUBATED WITH METHANOL

		Mg++	Mn++
No additions	22	39	56
DPN	40	55	67
ATP	128	125	125
DPN + ATP	114	113	114

Experimental conditions as in Table 1. Additions; 2 μm DPN; 8 μM ATP; 2.5 μM $MgCl_2$; 2.5 μM $MnCl_2$.
The values given represent the amount of formaldehyde accumulated in μg.

the homogenate was treated with Dowex—1 resin only very small amounts of formaldehyde accumulated during incubation with methanol when no further additions were made, but the addition of disphosphopyridine nucleotide and adenosine triphosphate gave the same values as were obtained in the homogenates which were not treated with Dowex—1 (Tables 2—3).

Addition of Mg^{++} or Mn^{++} ions increased the accumulation of formaldehyde when added alone or with diphosphopyridine nucleotide. Mn^{++} ions were slightly more active than Mg^{++} ions. When also adenosine triphosphate was added, a further addition of Mg^{++} or Mn^{++} had no effect (Table 3).

Because the effect of the nucleotide cofactors may also be due to the fact that they can act as substrate for liver xanthine oxidase,

TABLE 4

EFFECT OF THE ADDITION OF XANTHINE, ATP AND DPN ON THE ACCUMULATION OF FORMALDEHYDE IN DIALYSED RAT LIVER HOMOGENATES INCUBATED WITH METHANOL

Additions	Formaldehyde Accumulated μg
None	28
DPN	49
ATP	88
Xanthine	71

Experimental conditions as in Table 1. Additions: 5 μM xanthine; 2.5 μm DPN; 5 μM ATP.

the effect of the addition of xanthine on the accumulation of formaldehyde was studied in experiments recorded in Table 4. It is seen that in dialysed homogenates xanthine also clearly increased the accumulation of formaldehyde during incubation with methanol.

DISCUSSION

The increase in the amount of formaldehyde recovered from the incubation mixture when semicarbazide was present as a trapping agent can be taken as an indication that the amount of formaldehyde which is recovered without semicarbazide really represents only a fraction of the formaldehyde formed during the incubation. Most of it is metabolized further and in part also bound to proteins when no trapping agent is used. This is also seen from the earlier observation that the recovered formaldehyde represents only about 10 per cent of the methanol utilized during the same time (4).

However, the ratio between utilized methanol and accumulated formaldehyde in rat liver homogenates incubated with methanol has been found to be very constant in experiments where the same homogenate is used (4). When different homogenates have been used this ratio has varied between 5 and 20 per cent of the utilized methanol, aged homogenates accumulating more formaldehyde per utilized methanol. In view of this constant ratio the accumulation of formaldehyde also reflects very well the utilization of methanol in rat liver homogenates. Because the determination of formaldehyde is less tedious but more accurate and sensitive and has fewer sources of error than the determination of methanol, only the accumulation of formaldehyde has been determined in these experiments.

The failure of the cofactors to influence the accumulation of formaldehyde in fresh undialysed liver homogenates indicates that these homogenates contain enough of these cofactors for the reactions concerned. However, when aged and dialysed preparations are used the cofactor requirements were more clearly seen. The preparations were still able to carry out these reactions but to a considerably less extent. The accumulation of formaldehyde was not totally abolished even after treatment with Dowex—1 resin for the removal of cofactors.

The increase in the accumulation of formaldehyde caused by the addition of diphosphopyridine nucleotide was rather small. The role of liver alcohol dehydrogenase which is a diphosphopyridine nucleotide dependent enzyme (6), in the oxidation of methanol is not yet settled. Although pure crystalline alcohol dehydrogenase from horse liver does not react with methanol (8), more crude preparations have been found to reduce diphosphopyridine nucleotide in the presence of methanol, although rather slowly (4, 5). The small increase in the formation of formaldehyde in the presence of diphosphopyridine nucleotide does not indicate that diphosphopyridine nucleotide dependent enzymes play any essential role in the oxidation of methanol to formaldehyde. On the other hand, the comparatively small amounts of diphosphopyridine nucleotide added are known to be rather rapidly destroyed by the rat liver homogenate (7).

The activating effect of adenosine triphosphate on the accumulation of formaldehyde indicates the coupling of the oxidation of methanol to some energy-requiring reactions. Also the activation by Mg^{++} and Mn^{++}ions in the absence but not in the presence of adenosine triphosphate may have the same cause.

There is, however, a further possibility for adenosine triphosphate and diphosphopyridine nucleotide to take part in the oxidation of methanol. Methanol can at least *in vitro* be oxidised by the peroxidatically functioning catalase in systems which consist of a primary oxidizing system generating hydrogen peroxide, and of methanol and catalase (1—3). Xanthine oxidase can form such a primary oxidizing system using adenosine triphosphate and diphosphopyridine nucleotide as substrates (7). The effect of the latter cofactors on the metabolism of methanol might thus be due to the increased formation of hydrogen peroxide by this system. To obtain some information on this possibility the effect of the addition of xanthine on the accumulation of formaldehyde was studied. As can be seen from Table 4 xanthine is also a potent activator of these reactions. When the activating effects of adenosine triphosphate, diphosphopyridine nucleotide and xanthine are compared on an equimolar basis, it is seen that they are all of the same magnitude, which indicates the possible participation of catalase and xanthine oxidase on the oxidation of methanol in these experiments. On the other hand, the effects of adenosine triphos-

phate and diphosphopyridine nucleotide would be additive if their only role in these reactions were to serve as substrates for xanthine oxidase. The accumulation of formaldehyde has, however, been less when both diphosphopyridine nucleotide and adenosine triphosphate have been added than with adenosinetriphosphate alone. It is not very likely that the saturation limit of xanthine oxidase has been obtained in these experiments.

To elucidate further the role of xanthine oxidase in the metabolism of methanol, experiments have been made with molybdenum-deficient and with disulfiram-treated rats in whose tissues the xanthine oxidase activities are low. The results of these experiments will be reported in subsequent papers.

SUMMARY

The effect of various cofactors on the accumulation of formaldehyde in dialysed and Dowex —1 treated rat liver homogenates incubated with methanol has been studied.

The addition of adenosine triphosphate clearly increased the accumulation of formaldehyde. A smaller increase was found when diphosphopyridine nucleotide was added. When both adenosine triphosphate and diphosphopyridine nucleotide were added the increase was smaller than with adenosine triphosphate alone. Also the addition of xanthine increased the accumulation of formaldehyde.

Addition of Mg^{++} or Mn^{++}ions increased the accumulation of formaldehyde when added alone or with diphosphopyridine nucleotide. When also adenosine triphosphate was added further addition of Mg^{++} or Mn^{++} had no effect.

REFERENCES

1. Chance, B.: Acta chem. scand. 1947:*1:*236.
2. Keilin, D., and Hartree, E. F.: Proc. Roy. Soc. 1936:*B 119:*141.
3. Keilin, D., and Hartree, E. F.: Biochem. J. 1945:*39:*293.
4. Koivusalo, M.: Acta physiol. scand. 1956:*39:*suppl. 131.
5. Lutwak-Mann, C.: Biochem. J. 1938:*32:*1364.
6. Quibell, T. H.: Z. physiol. Chem. 1938:*251:*102.
7. Richert, D. A., Bloom, R. J., and Westerfeld, W. W.: J. Biol. Chem. 1957:*227:*523.
8. Theorell, H., and Bonnichsen, R.: Acta chem. scand. 1951:*5:*1105.